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SPECIAL LISTING

CURRENT CANCER RESEARCH

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NERVOUS SYSTEM TUMORS

JULY 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service ● National Institutes of Health ●
National Cancer Institute

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Investigator Index

I. ETIOLOGY AND EPIDEMIOLOGY OF NERVOUS SYSTEM TUMORS

A. INDUCTION OF NERVOUS SYSTEM TUMORS BY NITROSO COMPOUNDS

1. EXPERIMENTAL CEREBRAL TUMORS BY TRANSPLACENTAL ENU - LATENCY PERIOD AND PHENOTYPIC TRANSFORMATIONS

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OBJECTIVE: The administration of ENU in single dose i.v. to the mother rat on the 17th day of gestation determines the appearance of cerebral tumors in the 80% of the offspring, after a latency period. The tumors are represented mostly by gliomas and neurinomas. The earliest tumoral lesions have been observed in the paraventricular white matter in the 2nd month of extrauterine life. Our study is directed to the identification of the phenomena which accompany, both at the morphological and at the molecular level, the phenotypic tumoral transformation taking place during the latency period.

APPROACH: The investigation concerns the cerebral areas where the tumors will develop. It is carried out in newborn rats from mothers treated on the 17th day of gestation with a single dose of ENU i.v. The brains are studied by morphological, histochemical, cell kinetics, quantitative cytofluorimetric and in vitro culture techniques. These latter concern also fetal brain cells cultivated until the neoplastic transformation.

PROGRESS: So far, the results obtained are as follows: The first tumoral lesions have been identified in the paraventricular white matter on the 30th day of extrauterine life. They appear as glial cell hyperplasias, often recognizable only by evaluating the cell density per microscopic field. The histograms by cytofluorimetry of cellular DNA of the paraventricular white matter and medullary center show that most cells are diploid. Only few are tetraploid and no cell is more than tetraploid. No difference has been observed between treated and control rats until the 60th day. This finding does not explain the latency period, even though this has been halved after the present investigation. An explanation, however, comes from the cell kinetics investigation. The cell cycle time of the first tumoral lesions, evaluated by autoradiography in vivo, obtained with the high speed scintillation method and thymidine at low specific activity, is about 20 hours, while the labeling index is very low (3%).

2. NERVOUS SYSTEM-SPECIFIC CHEMICAL CARCINOGENESIS

Rajewsky, M. F., Kirstein, U., Ahrens, O., Nehls, P., Muller, R., University of Essen, Inst. fur Zellbiologie, *Hufelandstrasse 55, D4301 Essen, Nordrhein West., Federal Republic of Germany*

Oncogenesis in pre- and postnatal rat brain by the direct-acting pulse-carcinogen N-ethyl-N-nitrosourea (EtNU) provides a model system for the study of chemical carcinogenesis. A single pulse of EtNU specifically results in a high incidence of neuroectodermal neoplasms; the magnitude of the carcinogenic effect is a function of the developmental stage of the nervous system during which the EtNU-pulse is applied (high-carcinogenicity after exposure during pre- and early postnatal age; decreasing carcinogenicity with increasing age). Fetal rat brain cells transferred to cell culture after exposure to EtNU in vivo undergo malignant transformation after a sequence of phenotypic alterations (including e.g., reduced substrate adherence, morphological transformation, soft agar colony formation, increased growth rate, increased plasminogen activator activity). We intend to compare the probability of neoplastic transformation between different tissues and between phenotypically differing cell populations within a given tissue (brain), as a function of their proliferative and differentiated (developmental) state at the time of exposure to EtNU. In particular we intend to investigate whether the cellular capacity to recognize, eliminate, and repair carcinogen (EtNU)-modified DNA components is connected with the probability of neoplastic transformation; and whether enzymic excision capacity for defined, potentially mutagenic DNA lesions (such as O6-ethyldeoxyguanosine (O6-EtdGuo) varies according to the type and differentiated (developmental) stage of the target cells. These studies are, therefore, concerned with the enzymology of excision repair of ethylated bases in DNA (chromatin), and will compare, with the use of newly developed, highly sensitive immunological detection methods for O6-EtdGuo, the elimination rates of O6-EtdGuo from DNA as well as structurally different fractions of chromatin, in different rat tissues, neural and non-neural cell types, and during different stages of prenatal and postnatal development.

BIBLIOGRAPHIC REFERENCES: Laerum, O. D., Haugen, A., Rajewsky, M. F. (1979): Neoplastic transformation of rat neural cells in culture after exposure to ethylnitrosourea in vivo. In: C. Wigley, L. M. Franks (eds.): Neoplastic Transformation in Differentiated Epithelial Cell Systems in Vitro. London-New York: Academic Press, in press.

3. AN INVESTIGATION OF THE CARCINOGENIC ACTION OF NITROSAMIDES AND RELATED COMPOUNDS USING TISSUE CULTURE TECHNIQUES

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OBJECTIVE: To identify changes in cell properties which occur during nitrosamide-induced carcinogenesis in the rat brain and to investigate the relationship of these to malignant transformation.

APPROACH: Pregnant rats (inbred BD IX line) are injected with ethylnitrosourea (ENU) on the 15th or 16th day of pregnancy. This transplacental exposure results in the induction of cerebral gliomas in a large proportion of the offspring. Cultures are prepared from the brains of foetuses or cerebra of offspring at a number of times after exposure to ENU but before the appearance of a visible tumor. Control cultures are prepared from animals exposed transplacentally to buffer. The cultures are examined for several features found characteristic of cells cultured from gross gliomas, e.g., tumorigenicity in newborn syngeneic animals, specific cellular morphology (phase contrast microscopy), growth in agar, fibrinolytic activity, altered surface morphology (scanning electron microscopy). Particular attention is being paid to the possibility that these characteristics are acquired at different times following exposure to ENU.

PROGRESS: The sequential in vivo-in vitro approach has shown that the presence of malignant cells can readily be demonstrated about half-way through the latent period. However, potentially malignant cells which only become tumorigenic after many months in culture are already present two days after exposure to ENU. These results are consistent with a multistage process of carcinogenesis. Further results suggest that fibrinolytic activity precedes the ability to form colonies in agar. A more detailed investigation of the sequence of changes and their relationship to each other is now being undertaken.

4. INTERACTION OF N-METHYL-N'-NITRO-N-NITROSOGUANIDINE WITH CELLULAR COMPONENTS AND CHANGES OF BIOLOGICAL FUNCTIONS OF THE COMPONENTS

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OBJECTIVE: To reveal the mechanism of carcinogenesis of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

APPROACH: 1. Compounds used are alkyl derivatives of MNNG and its related compounds. 2. Modification of nucleic acids or proteins by those compounds is investigated to find their adducts and various compounds which influence binding. 3. Induction of differentiation of cultured mouse neuroblastoma N-18 cells by MNNG and other compounds is investigated. 4. Induction of various activities of enzymes such as alkaline phosphatase, LDH, leucine aminopeptidase and beta-glucuronidase in oat-cell-type cultured human lung carcinoma cells is investigated. 5. Changes in activities of nicotinamide methyltransferase and 1-methylnicotinamide oxidase in rat liver after injection of MNNG and other compounds are investigated. 6. Changes in the biological functions of cellular components such as chromatin after modification by MNNG and its derivatives are investigated.

5. EFFECT OF X-IRRADIATION ON THE NEUROCARCINOGENIC EFFECT OF ETHYL-NITROSOUREA (ENU)

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OBJECTIVE: To investigate whether X-irradiation has a synergistic or an inhibitory effect on tumors of the nervous system produced by ENU.

APPROACH: Newborn rats are injected with ENU during the first 24 hours of life and during the second 24 hours of life are irradiated.

6. EARLY CHANGES IN POTENTIAL BRAIN TUMOR TISSUE

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Ethylnitrosourea (ENU) is a powerful transplacental carcinogen. A single dose to a pregnant rat produces neural tumors in adult offspring many months later. The long latency period offers an opportunity to look for early oncogenic changes in target neural tissues, applying a combination of in vivo and in vitro techniques.

We have demonstrated that tumorigenic doses of ENU produce chromosome aberrations in bone marrow and fetal tissues of treated animals as well as in cultured cells in vitro, suggesting that a mutation could be an indication, and possibly an explanation, for the initial oncogenic effect.

Rat neural tumors produced by ENU have been grown in cell culture. In cell lines from the gliomas and Schwannomas we have compared chromosome constitution, growth characteristics in culture, histological findings relating to the tumor and tumorigenicity of the cells. In 12 tumor lines excess chromosome 4 was present as a common chromosome finding, suggesting that extra chromosome 4 can be useful as a marker for ENU neural tumors.

Our main objective is to study early stages of oncogenesis in neural target tissues. Brain and trigeminal nerve ganglia are removed from rats treated transplacentally with ENU. At various stages postnatally neural tissues are initiated in cell culture and monitored for oncogenic transformation, cytogenetic changes and tumorigenicity.

We will attempt to determine whether excess chromosome 4 is related to ENU as a specific etiologic agent by studying the chromosome constitution of non-neural ENU tumors, or whether the chromosome is associated with a particular tumor cell type, by studying rat brain tumors induced by other agents. The role of chromosome 4 in initiation or progression of the tumors will also be investigated by examining direct preparations of tumors with special emphasis on a search for competing cell lines and subclones.

7. NEURO-ONCOGENESIS BY RESORPTIVE CARCINOGENS

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Alkylnitrosoureas, because of their chemical simplicity and known catabolism as well as their ability to induce a high incidence of neurogenic tumor with precision, provide an excellent opportunity to study the basic problem of neoplastic transformation. It is proposed to examine the formation and removal of O6-alkylguanine as a possible DNA lesion crucial to neoplastic transformation. Attempts will be made to correlate alterations in DNA and RNA synthesis and the metabolism of non-histone chromosomal proteins induced by methylnitrosourea and ethylnitrosourea with the carcinogenic potential of these compounds. Gene activation associated with tumor promotion will also be studied in relation to proteolytic derepression by the chromosomally associated serine-protease. Induction of the synthesis of helix destabilizing protein and its control at the level of transcription or translation will be investigated as the process responsive to both tumor promotor and chemical carcinogen.

The precision of ethylnitrosourea in the neurogenic tumor induction will further be utilized in evaluating the role of nerve growth factor in suppression or regression of neoplastic transformation. The recently discovered thymic lymphoma production in rats exposed to methylnitrosourea will be studied in pursuit of establishing the tumor model for future biochemical, immunological and therapeutic investigations.

8. STUDIES IN NEURO-ONCOGENESIS AND NEUROGENESIS

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The proposed investigation is designed to elucidate the effects of transplacental administration of N-ethyl-N-nitrosourea (ENU) on the postnatal development of rodent cerebellum and hippocampus. Since ENU-treated animals may develop tumors of the cerebellum and hippocampus, and tumorigenic incidence is highest during morphogenesis, this proposal hypothesizes that detectable developmental errors may be produced that might result in a predisposition to neuro-oncogenesis. A major emphasis will be placed on morphology, and several research methods (histology, histochemistry, electron microscopy) will be utilized; whenever possible, quantitative and stereologic analyses will be included. Autoradiographic studies of cell proliferation and differentiation, as well as the localization and fate of 14C-ENU, will also be conducted and correlated with structural observations. This research will delineate the teratogenic action of ENU, define the role of neurogenesis during chemical carcinogenesis, and explore the etiology and pathogenesis of brain tumors.

BIBLIOGRAPHIC REFERENCES: Zagon, I.S., and P.J. McLaughlin, 1978. Transplacental exposure to ethylnitrosourea and its effects on body, brain, and cerebellar development in the rat. Cancer Res. 38: 1263-1268. Zagon, I.S., and C.L. Schengrund. 1978. Neuronal and non-neuronal properties of neuroblastoma cells. Exp. Cell Res. 114: 159-165.

9. X-RAY MODIFICATION OF ETHYLNITROSOUREA TRANSPLACENTAL CARCINOGENESIS

Kalter, H., Warkany, J., Childrens Hospital, *561 S. 17th St., Livingston Pike, Columbus, Ohio*, 43205, United States of America

Pregnant Sprague-Dawley rats were X-irradiated on the 16th day of gestation with 5-250 rad and injected intraperitoneally 4 days later with 10 mg/kg ethylnitrosourea (ENU). The offspring were observed over their life span for the appearance of neurogenic tumors. The frequency of animals that developed tumors surviving beyond 4 weeks of age was inversely related to X-ray dosage: a) about 15% after exposure to the largest doses; b) 39-46% after the intermediate ones, and c) 58-65% after the smallest. After treatment with ENU alone, 69% occurred. The reductions in tumor frequency were not due to increased mortality rate of tumor-prone animals either before or after the onset of tumor appearance. Mean offspring weight at 4 weeks and 4 months of age was inversely related to X-ray

dosage but the weight of those animals developing tumors later was not significantly different from that of animals remaining tumor free. Mean time of tumor appearance and mean number of tumors per affected animal were unrelated to tumor frequency. Destruction of target cells does not sufficiently explain the results.

B. VIRUSES AND NERVOUS SYSTEM TUMORS

10. ASSOCIATION BETWEEN PRIOR POLIOMYELITIS AND CENTRAL NERVOUS SYSTEM TUMOURS

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Two cases of spinal cord tumours in persons who had contracted poliomyelitis 30 years previously were reported by us (Lancet, 1, 823, 1975). Since then we have identified a further case. It is proposed to study this relationship further.

11. CHRONIC CENTRAL NERVOUS SYSTEM DISEASE STUDIES - SLOW, LATENT AND TEMPERATE VIRUS INFECTIONS

Gajdusek, D. C., Gibbs, C. J., Amyx, H. L., Aoki, T., Asher, D. M., Bahmanyar, S., Borras, M., Brown, P. W., Garruto, R., Gourmelon, P., Kingsbury, D. T., Kuroda, Y., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Neurological & Communicative Dis & Stroke, Laboratory of Central Nervous System Studies, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, parkinsonism-dementia, Parkinson's, Pick's and Alzheimer's diseases, Huntington's chorea, supranuclear palsy, other presenile dementias, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, SSPE, PML, dialysis encephalopathy, and intracranial neoplasms. Even familial, apparently hereditary, diseases may be slow virus infections. Subacute spongiform virus encephalopathies (kuru and Creutzfeldt-Jakob (CJD) diseases of man; scrapie and mink encephalopathy) are caused by unconventional viruses with unique properties posing important theoretical problems to microbiology and molecular biology; a major goal is elucidation of their structure and mechanisms of replication. Transmissible virus dementias are increasingly recognized worldwide as causes of death: high incidence foci, transmission by corneal transplant or brain surgery, and occupational hazards from exposure to brain occur. In order to determine the usual mode of infection with the virus, a worldwide epidemiological study of transmissible virus dementia (CJD) cases is under way with special attention to familial clusters of cases and with a quest for possible relationship of scrapie of sheep to the human disease.

BIBLIOGRAPHIC REFERENCES: Baringer, J.R., Gajdusek, D.C., Gibbs, C.J., Jr., Masters, C.L., Stern, W.E., and Terry, R.D.: Transmissible dementias: current problems in tissue handling. Neurology, 1979. In press. Benfante, R., Hoffman, P.M., Garruto, R.M., and Gajdusek, D.C.: HLA phenotypes in the Chamorro population of the Mariana Islands. Human Biology 51: 201-212, 1979.

12. STUDIES ON THE TUMOR INDUCING PROPERTIES OF THE PAPOVAVIRUSES JC AND BK

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The purpose of this research project was to study what factors JC and BK viruses possess which determine what type of brain tumor is formed. When JC virus, for example, is inoculated into the right hemisphere of the newborn hamster brain a variety of tumors including medulloblastoma, glioblastoma, ependymoma and pinealoma will be formed. BK, on the other hand, will form ependymomas and choroid plexus papillomas only. The first part of our grant request was to inoculate high titer purified preparations of these viruses into highly specific sites of the hamster brain using a stereotactic mold developed in this laboratory. Preliminary studies in this regard have shown that there is a diffusion of virus beyond the site of local injection which to date has been difficult to control. We are continuing efforts to purify the virus sufficiently for these studies to proceed with this during the coming year.

Efforts in our laboratory, however, have focused on the second part of our research which concerns in vitro transformation of neuroglial cells and study of incorporation of virus genome into their nucleic acid. For this study we have succeeded in maintaining monolayer cultures from a variety of regions of the newborn Syrian hamster brain. At present we have cultures of cerebral and cerebellar hemisphere, cerebellar vermis, brainstem and choroid plexus. In addition, we have succeeded in preparing large quantities of highly purified JC, BK and SV40 viruses. Purification of JC virus has been particularly difficult since it has required growth on cultures of human fetal neuroglia. Cultures have been inoculated and are now being maintained with the expectation that transformation to malignant cell type will occur within the next three months. When such transformation has taken place the individual cell types will be studied histologically and by electron microscopy to determine whether specific types of tumor cells have been induced by the viruses.

13. HUMAN CANCER SEROLOGY

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The two objectives of the Human Cancer Serology Program are as follows: I. Serological definition and biochemical characterization of distinctive cell surface antigens of human cancer. II. Immunovirologic analysis and biochemical characterization of human cancers of suspected viral etiology. The proposed studies relate to two central questions concerning human cancer: Do human tumor-specific antigens exist and do viruses play a role in the etiology of human cancer? The technique of autologous typing of malignant cells will be used to analyze cell surface antigens of malignant melanoma, renal cancer, astrocytoma, leukemia, lymphoma, and ovarian cancer, and will be applied to breast, lung, and colon cancer as improved methods for the in vitro propagation of these cancers are developed. Serological definition of tumor-specific cell surface antigens of human cancer will be followed by: a) Genetic analysis to determine the chromosomal loci specifying these antigens, b) search for natural antibody to these antigens in the normal human population, c) biochemical characterization, d) assessment of the clinical significance of humoral immunity to these antigens in patients with cancer, and e) development of maximally immunogenic forms of cancer vaccines. Immunovirological studies will focus on selected human cancers that are suspected on the basis of serological, virological, and epidemiological evidence to be related to herpesviruses or papovaviruses. The following associations will be investigated: The relation of cytomegalovirus to Kaposi's sarcoma, genital cancer, and colon cancer; the relation of Epstein-Barr virus to nasopharyngeal cancer, and the relation of BK and JC papovaviruses to brain cancer, renal cancer, and cancers developing in immunodeficient patients. Using a variety of serological assays and reference viral antigens and antibodies to identify the range of herpesvirus and papovavirus antigens, evidence will be sought for the presence of viral antigens in human cancer cells and for the occurrence of corresponding antibody in patients with cancer as compared with the normal population.

14. PATHOGENESIS - SLOW VESICULAR STOMATITIS VIRUS INFECTION

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This proposal will focus on the following objectives: (1) To define the in vivo capacity of ts G41 infection in BALB/c mice to produce persistent or chronic CNS viral infection. (2) To study the neurovirulence of various clonal isolates of ts G22 and ts G31 by means of cloning brain and spinal cord isolates and testing representative plaque purified clones with respect to plaque morpnology and size, growth kinetics in neuroblastoma and BHK-21 cells at 31 degrees, 37 degrees and 39 degrees C and in vivo clinical and histopathological behavior. (3) To study the behavior of wt and selected ts VSV mutants in neurally derived cell lines. Cell lines to be used include N2A, N-18 (murine neuroblastoma), C-6 rat glioma and ependymoma cells. Studies include growth kinetics and mechanisms of maturation

and replication in vitro using light, transmission and scanning electron microscopy. (4) To define the in vivo subcellular localization of major VSV antigens, i.e. G, M, N proteins, in infected neurons in vivo by means of an immunoperoxidase technique. (5) To study additional ts VSV mutants belonging to complementation groups II and III. Since the slowly progressive CNS infection and spongiform myelopathy are seen with Glasgow mutants belonging only to complementation groups II and III, these studies will determine whether the production of spongiform myelopathy is a general property of certain complementation groups or unique to individual mutants. (6) To define the in vivo capacity of Chandipura virus and selected ts mutants of this human rhabdovirus to produce CNS disease. (7) To define the in vivo interaction occurring between homologous DI particles and ts VSV mutants. (8) Biochemistry: A major segment of the proposed study will involve the characterization of the growth kinetics and RNA and viral protein synthesis of the original cloned temperature-sensitive VSV mutants ts G22 and G31 that produced the prolonged CNS disease and spongiform neuropathology. As a result, a comparative biochemical study of these clonal isolates as well as the original mutants will be one of the major focuses of the proposed research. (Text Abridged.)

15. RELATIONSHIP BETWEEN HUMAN PAPOVAVIRUSES AND TUMORS OF THE NERVOUS SYSTEM (ABBREV)

Beth, E., Giraldo, G., Mike, V., Chernesky, M. A., Huraux, J. M., Claudio, F., Giordano, G., Gharbi, R. M., Memorial Sloan Kettering Cancer Center, Immunovirology Section, *1275 York Ave., New York, New York*, 10021, United States of America

Human papoviruses, particularly JC virus, can transform hamster and rat cells in vitro and can induce tumors in vivo when injected in hamsters and monkeys. The JC virus induces mainly tumors of the nervous system, and has been clearly shown to be the etiological agent of progressive multifocal leucoencephalopathy (PML), a demyelinating disease. The main objective of the project is to establish an association between the above mentioned viruses and malignancies of the nervous system by extensive sero-epidemiological studies. The immunovirological antibody profile against early and late antigens determined by papovaviruses is established in sera of cancer patients with tumors of the nervous system from USA, Europe, and North Africa. Two sex- and age-matched (2-5 years) healthy controls, living in the same areas and in similar socio-economic conditions, are used for each patient. A second control group will consist of serum samples from age-and sex-matched patients with a cancer of different origin and location but similar therapy.

16. BIOLOGICAL STUDIES OF VARIOUS NORMAL AND VIRUS-INFECTED MALIGNANT CELLS

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It is the primary purpose of this project to study some of the pertinent factors which influence cell differentiation and malignant transformation, using techniques and approaches which range from the microscopic to the molecular level. Particular emphasis is given to those systems in which murine RNA tumor viruses or their precursors may be the transforming agent. A variety of mouse model systems are used, including plasma cell tumors, mammary tumors, and neuroblastomas. Current projects include: 1) Characterization of intracisternal A particles (IAP) with particular emphasis on homology to known RNA tumor viruses, molecular events associated with replication, conservation and divergence of IAP genes, and factors controlling the expression of IAP genes; 2) effects of interferon on the assembly and maturation of murine oncornaviruses with special emphasis on the study of mechanisms whereby whole virions are rendered noninfectious.

BIBLIOGRAPHIC REFERENCES: Chipelinsky, A., Gantt, R., and Wivel, N.: Presence of RNA methylases in intracisternal A particles purified from a mouse plasma cell tumor. Eur. J. Biochem. 103:339-347, 1980. Pitha, P.M., Fernie, B., Maldarelli, F., Hattman, T., and Wivel, N.A.: Effect of interferon on mouse leukemia virus (MuLV). V. Abnormal proteins in virions of Rauscher MuLV produced in the presence of interferon. J. Gen. Virol. 46: 97-110, 1980.

17. PAPOVAVIRUSES IN NONHUMAN PRIMATES

London, W. T., Palmer, A. E., Madden, D. L., Houff, S. A., Gravell, M., Wallen, W. C., Sever, J. L., Curfman, B. L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Neurological & Communicative Dis. & Stroke, Experimental Pathology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Seventy owl monkeys (Aotus triviragatus) were inoculated with JC virus, a human polyomavirus. An additional 22 monkeys were used as controls. Some of the animals were used to confirm our finding that two owl monkeys inoculated with JC virus developed brain tumors at 16 and 25 months postinoculation, respectively. Remaining animals were used in passage of the JC induced primary owl monkey tumor and variable routes of inoculations (single routes) instead of the multiple route in the original studies. The animals have been monitored for JC antibody. To date, most the virus inoculated animals have developed hemagglutination inhibition (HI) titers to JC virus.

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18. ETIOLOGY, IMMUNOLOGY AND BIOLOGY OF BRAIN TUMORS

Bigner, D. D., Duke University, School of Medicine, Dept. of Pathology, *Box 3711, Durham, North Carolina*, 27710, United States of America

Our objectives are to investigate basic aspects of brain tumors (especially gliomas) in etiological, immunological, tumor biological, and experimental chemotherapeutic areas. In etiological studies we will seek the association of retroviruses and human papovaviruses with spontaneous human or animal gliomas; establish the transplacental neuro-oncogenicity of acrylonitrile; study genetic susceptibility of mice to neuro-oncogenetic viruses and chemical carcinogens; and use banding and non-banding cytogenetic techniques to guide etiological studies of animal and human gliomas. In immunological studies, we will purify and characterize antigens associated with spontaneous human and mouse gliomas, and determine if quantitation of these antigens can be used to monitor tumor size, and as an adjunct in diagnosis. In tumor biological studies, we will establish whether or not the phenotypic heterogeneity of neoplastic cells in human gliomas with respect to morphological, biochemical, and immunological differentiation extends to chemotherapeutic sensitivity, as tested in athymic mice; and whether endothelial cells of human and experimental gliomas are neoplastic. We will develop and use a glioma animal model in gerbils to test intracarotid chemotherapy; and we will develop reliable animal models of medulloblastoma and craniopharyngioma in athymic mice.

19. MORPHOLOGY OF CHRONIC VIRAL INFECTIONS OF THE BRAIN

Zu Rhein, G. M., University of Wisconsin, Madison Campus, Medical School, Dept. of Pathology, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

In the proposed project our objectives are: 1. To examine the oncogenic potential of JC papovavirus which has been isolated from human brains with progressive multifocal leukoencephalopathy and also from the urine of immunologically impaired or immunosuppressed patients. After inoculation by various routes, gross autopsies and light-microscopic studies will be performed on Syrian hamsters, which have been found previously to be highly susceptible to JC virus tumor induction. Selected neoplasms will be studied by electron microscopy. Sequential details of JC virus induced medulloblastomas will be studied by light and electron microscopy and by immunofluorescent technique. Following a positive pilot study, JC viral oncogenicity will be examined more extensively in a series of owl monkeys, with emphasis on the central nervous system. 2. To conduct a first test concerning possible teratogenic effects of JC virus in the Syrian hamster. 3. To extend our search in

current human brain autopsy material for diseases of slow viral nature.

20. FELINE MODEL OF OCULAR MELANOMA

Albert, D. M., Essex, M. E., Sang, D. N., Harvard University, Boston Campus, Massachusetts Eye & Ear Infirmary, Howe Laboratory, *243 Charles St., Boston, Massachusetts*, 02114, United States of America

A study is proposed for the development and characterization of a feline model of human ocular melanoma. Preliminary experiments indicate that injection of a naturally occurring feline sarcoma virus into the eyes of kittens results in the development of uveal melanomas in a high percentage of animals. These tumors bear a close clinical and morphological resemblance to human ocular melanomas. Use of this virus provides the first experimental virally induced melanoma in animals. Tumors thus far developed show a spectrum of cell types consistent with the Callender classification used to categorize human melanomas, and varying degrees of pigmentation. This application proposes to define the developmental events occurring as pigmented cells in the eye undergo malignant transformation, and trace the course of these tumors. The model would appear applicable for the testing of new diagnostic and therapeutic techniques which could be adapted for use with human tumors. This is, in addition, a virus of interest and significance with regard to feline neoplastic and non-neoplastic diseases, and the ocular model offers opportunities for further investigation of the mechanism of natural immunosurveillance against tumors, the immunological response of the host to ocular infection and neoplasia, and the response to immunotherapy.

21. PATHOGENESIS OF VIRUS-INDUCED GLIOMA (RATS)

Wollman, R. L., University of Chicago, Pritzker School of Medicine, Dept. of Medicine, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

This is part of a broader project. A summary of this subproject is not available.

22. ONCORNAVIRUS INFECTION OF TRIGEMINAL GANGLION

Tenser, R. B., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Microbiology & Cell Biology, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

This is part of a broader project. A summary of this subproject is not available.

C. EPIDEMIOLOGY OF NERVOUS SYSTEM TUMORS

1. EPIDEMIOLOGICAL STUDIES OF OCULAR TUMORS

23. THE GENETICS AND EPIDEMIOLOGY OF RETINOBLASTOMA

Draper, G. J., Sanders, B. M., University of Oxford, Dept. of the Regius Professor of Medicine, *S. Parks Rd., Oxford OX2 6HE, England, United Kingdom*

The objectives of this study are: (1) To determine the incidence of second primary tumors, particularly osteosarcoma, among children surviving retinoblastoma; (2) to determine whether there is an increased incidence of other cancers, either in general or of certain specific types, among the parents of children with retinoblastoma; (3) to ascertain whether, as for certain congenital defects, the incidence of this disease is related to parental age; and (4) to examine associations between congenital defect, particularly mental retardation, and retinoblastoma. About 500 case of retinoblastoma have been ascertained through hospital, cancer registries and death certificates; epidemiological and familial data are also being collected.

24. SPORADIC RETINOBLASTOMA IN RELATION TO PARENTAL AGE AND SEASONAL VARIATION

Matsunaga, E., Minoda, K., National Inst. of Genetics, Dept. of Human Genetics, *Shizuoka-ken, Yata 1111, Mishima 411, Japan*

Follow-up investigations of progeny of survivors of sporadic retinoblastoma revealed that they comprise two etiologically distinct groups, hereditary and non-hereditary. All bilateral cases belong to the hereditary group and they are due to dominant germinal mutations, while the majority (90-95%) of sporadic unilateral cases are non-hereditary, and the cause appears to be the same mutational event in the somatic (retinoblast) cells. Although a few investigations (including ours) suggest that paternal age may affect the occurrence of sporadic bilateral but not unilateral cases, the evidence is as yet not very convincing. We intend to clarify this point, together with the possible effect of seasonal variation upon the presumed somatic mutations. Data concerning about 400 sporadic cases of retinoblastoma which occurred in from 1970 to 1975 in Japan have already been analyzed, and new cases (approximately 100 per year) will be collected over the next several years.

25. STUDY OF RETINOBLASTOMA IN JAPAN AS A POSSIBLE POPULATION MONITORING SYSTEM FOR ENVIRONMENTAL MUTAGENS

Sugahara, T., Nikaido, O., Nakatsugawa, S., Kyoto University, School of Medicine, Dept. of Experimental Radiology, *Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606, Kyoto, Japan*

Because an increasing number of environmental mutagens have been detected by laboratory screening methods, the need to monitor human population directly for mutational risk has been emphasized by many geneticists. Recent studies by Knudson offer hope that certain malignant neoplasms might be used to assess somatic mutation rates. According to Knudson, the age at first diagnosis of these diseases may be related to the annual somatic mutation rate. Thus the data on the age at first diagnosis of unilateral and bilateral retinoblastoma have been collected throughout Japan. Since 1975 the data have been obtained by a nationwide registry of the disease in cooperation with the Ophthalmology Society of Japan. A first report suggesting an increase in mutation rate in Japan has been published (Mutation Research 30, 137, 1975). The study continues.

26. NATIONAL REGISTRATION OF CHILDREN WITH RETINOBLASTOMA IN JAPAN

Minoda, K., University of Tokyo, Branch Hospital, Dept. of Ophthalmology, *3-28-6 Mejirodai, Bunkyo-ku, Tokyo 112, Tokyo, Japan*

Retinoblastoma is the most common intraocular malignant tumor in Japan. To investigate epidemiology, genetics, pathology and clinical aspects of retinoblastoma in Japan, the national registration of retinoblastoma children in Japan was started in 1975 by the Committee for National Registration of Retinoblastoma Children, consisting of 44 ophthalmologists and three other scientists. Interim reports have appeared in Acta Soc. Opthalmol. Jpn. 80: 1648, 1976, and in the proceedings of the International Symposium on Retinoblastoma, Jpn. Opthalmol. 22, No. 3, 1978.

27. RETINOBLASTOMA IN FINLAND SINCE 1912 - INCIDENCE OF OTHER MALIGNANT NEOPLASMS IN RETINOBLASTOMA PATIENTS AND THEIR RELATIVES

Tarkkanen, A., Merenmies, L., Helsingin Yliopisto, Eye Hospital, *Haartmaninkatu 4, 00290 Helsinki 29, Finland*

It has been suggested that retinoblastoma might be used as an index cancer to whose frequency the frequency of other cancers could be compared. Hence efforts should be made to bring to light data on this cancer from different countries of the world. It has also been shown that bilateral retinoblastoma survivors may be more likely to develop another primary malignant neoplasm than any other cancer patients. It is proposed to estimate the incidence of other malignant neoplasms in retinoblastoma patients and their relatives. The data will be collected from Finland, with the pathology laboratory of the Helsinki University Eye Hospital serving as the main source of information.

28. EPIDEMIOLOGY OF RETINOBLASTOMA IN NEW ZEALAND

Suckling, R. D., Fitzgerald, P. H., Clemett, R. S., Christchurch Hospital, Dept. of Ophthalmology, *Private Bag, c/o Christchurch Clinical Sch., Christchurch, New Zealand*

OBJECTIVES: To continue recording all cases in New Zealand since 1948 to determine presence or absence of clustering, prognosis, and, eventually, the number of inherited cases.

New Zealand ophthalmologists are requested to report all cases. (Cancer statistics, births and deaths records are used to check.)

29. HISTORICAL REVIEW OF MALIGNANT MELANOMA IN ALBERTA 1967-1976

Birdsell, J. M., Jerry, L. M., Grace, M. G., Gunther, B. K., Southern Alberta Cancer Centre, Dept. of Epidemiology, *2104 2nd St. S.W., Calgary, Alberta,* T2S 1S5, Canada

Records of all patients diagnosed as having melanoma of skin or eye during the past ten years were identified from the Alberta Cancer Registry. These files were reviewed and information collected on sex, age, residence history, method of diagnosis, site, pathological features, stage at diagnosis, duration of stages, sites of metastases, treatment and survival. Analysis of data on 99 ocular melanoma patients showed that ocular sites constituted 16% of all melanomas for that period, and 70% of ocular malignancies were melanomas. Female patients had a much higher incidence of the more malignant mixed cell type tumors than males, while the converse was true of the less malignant spindle cell melanomas. Within each cell type, females survived significantly longer than males. The actuarial five year survival rate of males and females combined was 62%. Metastases occurred in 29 of the 99 patients, with liver being the sole or concomitant initial site of metastases in 22 of these cases. There was no improvement in survival rates over the ten-year period. Analysis of data of skin melanoma patients is underway and will provide information on the incidence, treatment given, recurrence and survival rates and the correlation of various factors with prognosis.

30. CANCER IN THE IGBOS OF NIGERIA

Onuigbo, W. I., Medical Foundation, *8 Nsukka Ln., Uwani, P.M.B. 1098, Enugu, Nigeria*

This ongoing study of the relative frequency and the characteristics of cancer cases biopsied in different hospitals serving the Igbos of Nigeria and examined personally has resulted in the publication of data on tumors of the skin, eye, vulva, cervix, ovary, testis, alimentary tract, liver, oral cavity, lip and jaw. The most recent publications on tumors are: Schistosomiasis of ovarian thecoma (Med. J. Zambia, 12, 27-28, 1978) and adolescent breast masses in Nigerian Igbos (Amer. J. Surg., 137, 367-368, 1979). Work continues.

31. EPIDEMIOLOGY AND PATHOLOGY OF CANCER IN MALAWI

Hutt, M. S., Burkitt, D. P., Borgstein, J. I., Tozer, R. S., Molyneux, M., Cook, P., Lucas, S., University of London, St. Thomas Hospital Medical School, Dept. of Morbid Anatomy, *Lambeth Palace Rd., London SE1 7EH, England, United Kingdom*

All pathological specimens from Malawi for the last 10 years have been examined in St. Thomas' Hospital Unit of Geographical Pathology. Analyses of the epidemiological features of Kaposi's sarcoma and of precancerous and cancerous lesions of the conjunctiva have been completed; studies of salivary gland neoplasms, malignant histiocytosis and solitary myeloma are under way.

32. CANCER EPIDEMIOLOGY IN TANZANIA, EAST AFRICA

Henschke, U., Luande, J., Muindi, J. N., Hiza, P. R., Phillip, J., Shaba, J., Mgaya, M., University of Dar es Salaam, Muhimbili Medical Centre, Dept. of Radiology & Radiotherapy, *P.O. Box 3592, Dar es Salaam, Tanzania*

Epidemiological studies, based on the national registry and on the registry of pathological specimens, including case studies of the most frequent cancers in Tanzania (cervix uteri, liver, skin in albinos, and stomach) are envisaged. Investigations of cancers which appear to be unusually frequent in relative terms (Burkitt's lymphoma, Kaposi's sarcoma, retinoblastoma) as well as of the cancers rare in East Africa, but frequent in Europe and North America (lung, colon, breast, pancreas, prostate) are contemplated.

33. RISK FACTORS IN MALIGNANT MELANOMA OF CHOROID AND CILIARY BODY

McLean, I. W., Zimmerman, L. E., U.S. Dept. of Defense, Armed Forces Inst. of Pathology, *c/o Walter Reed Medical Ctr., Washington, District of Columbia,* 20306, United States of America

The purpose of this long-range project is to ascertain risk factors in malignant melanomas of the choroid and ciliary body.

During the past year, work was begun on three new studies. The first is to compare factors influencing patient survival in those having tumors with and without invasion of the vortex veins of the eye. The second is to determine the relative importance of the risk factors as a function of time following enucleation. The third is to develop a mathematical model to describe the time course for the metastatic process. The model will enable us to plan better studies to test the varying hypotheses that have been proposed for the mechanism by which uveal melanomas metastasize. All of these are long-term projects needing approximately 4,000 cases with follow-up data for statistically valid results. To date, 2,300 cases have been coded with follow-up for computer retrieval. A Fortran program has been written for the mathematical model and is being tested.

BIBLIOGRAPHIC REFERENCES: Zimmerman, L.E., and McLean, I.W.: Metastatic Disease from Untreated Uveal Melanomas. Am. J. Ophthalmol. 88: 524-534, 1979. Zimmerman, L.E., and McLean, I.W.: An Evaluation of Enucleation in the Management of Uveal Melanomas. Am. J. Ophthalmol. 87: 741-760, 1979.

34. CASE-CONTROL STUDY AND OPHTHALMOLOGY SURVEY OF PLANT IN CHARLESTON, W. VA. - NCI

Egan, E., Smith, L., Jones, U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance, Hazard Evaluations & Field Studies, *4676 Columbia Pkwy., Cincinnati, Ohio,* 45226, United States of America

In response to an employee request, NIOSH began an investigation into an alleged cancer hazard at a DuPont chemical plant in Belle, West Virginia. Subsequent to this employee request, NIOSH received a completed report of a DuPont epidemiologic study that demonstrated an excess risk of cancer of the eye, kidney and lung among employees at the DuPont Belle plant.

In order to thoroughly investigate the causes of this excess cancer risk identified among employees at the DuPont Belle plant, NIOSH proposed to conduct a cross-section ophthalmologic survey of workers at DuPont Belle plant and a case control study of the eye and kidney cancer cases.

The cross-section ophthalmologic survey was conducted from March 13, 1978, to April 21, 1978. A total of 1153 persons were examined. One additional case of ocular melanoma was discovered in a pensioned employee. A follow-up of persons discovered in the course of the survey to have pigmented lesions of the optic fundus is planned for FY 80.

The work histories of all the eye and kidney cancer cases identified at the DuPont Belle plant will be compared to the work histories of a group of matched controls to determine if the cancer cases worked more frequently than the controls in any specific job areas.

35. INCIDENCE OF UVEAL MALIGNANT MELANOMA IN MINNESOTA

Wilkes, S., Mayo Foundation, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

This is part of a broader project. A summary of this subproject is not available.

2. EPIDEMIOLOGICAL STUDIES OF NERVOUS SYSTEM TUMORS IN CHILDREN

36. EPIDEMIOLOGY OF CHILDHOOD CANCER IN THE PROVINCE OF TORINO, ITALY

Pastore, G., Anglesio, E., Terracini, B., Universita degli Studi di Torino, Clinica Pediatrica, Serv. di Oncologia, *Piazza Polonia 94, c/o Ospedale Regina Margherita, 10126 Turin, Italy*

A special childhood cancer registry (children aged 0-14) for residents in the province of Torino (total population at the time of the 1971 census: 2,287,016, of whom 497,223 were aged 0-14) has been operating since 1965. Cases are recorded according to ICD and histological types. Data for the 1967-74 period have been published (Rivista Italiana di Pediatria 5: 337-345, 1979). The collection of cases and their analysis have been completed for the period 1975-78. Incidence rates are similar to those of other industrialized countries in the Western World.

Incidence rates have been calculated for residents in the city of Torino and residents in the rest of the province as well as for different areas within the province. The incidence rate for all cancers and for neurogenic tumors was higher in the city of Torino than in the rest of the province in the period 1967-74, but not between 1975 and 1978. By 1982 collection of cases will be completed for the period up to December 1981. Trends in incidence, mortality and lethality are being investigated, as well as the distribution of cancer in children among the indigenous population as compared to the population immigrating from other parts of Italy.

37. EPIDEMIOLOGY OF CHILDHOOD MALIGNANCIES IN JAPAN

Hirayama, T., Ministry of Health & Welfare, Epidemiology Div., *Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Tokyo, Japan*

Patterns of occurrence of childhood malignancies in Japan are being studied in relation to selected risk factors based on data from nationwide registration. A total of 10,575 children (5,951 males; 4,624 females) under age 15 were registered between 1969 and 1977. Malignancies included leukemia (4,607), malignant lymphoma (750), neuroblastoma (1,002), Wilms' tumor (485), liver tumor (230), retinoblastoma (892). Age-specific incidence rates were calculated for each. When compared with vital statistics figures for all Japan, maternal ages had little or no influence, but significant risk elevation was noted under age three with increase in weight at birth, for leukemia, neuroblastoma, Wilms' tumor, testis tumor, and teratoma. Abdominal radiation exposure during the later part of pregnancy was noted to enhance leukemia risk in those children when compared with 1,011 controls of similar age. Down's syndrome was frequent in leukemia cases under age three and many cases of congenital malformation, including seven cases of hemihypertrophy, five of aniridia, six of congenital heart disease, were recorded co-existent with Wilms' tumor. The mean ages for retinoblastoma were five, six and seventeen months for bilateral, unilateral with family history, and unilateral without family history cases respectively. Analysis continues.

38. CHILDHOOD CANCER IN EAST JAVA

Hoediasmoro, D. S., Universitas Airlangga, Faculty of Medicine, Dept. of Pathology, *Jalan Dharmahusada No. 47, Surabaya, East Java, Indonesia*

This is a relative frequency study of childhood cancer (between 0-12 years of age) found in the Department of Pathology, Faculty of Medicine, University of Airlangga in Surabaya. Most cases in this Department are from the East Java area. Age and sex distribution and geographical variations are to be examined, and the possible correlation with socioeconomic conditions will be investigated. Special attention will be given to malignant lymphoma and retinoblastoma. Information on socioeconomic conditions will be obtained by interview.

39. LONG-TERM FOLLOW-UP OF PERSONS EXPOSED TO SCALP IRRADIATION IN CHILDHOOD

Modan, B., Ron, E., Chaim Sheba Medical Center, Dept. of Clinical Epidemiology, *Bitan 27, Tel Hashomer, Ramat Gan, Israel*

Approximately 11,000 individuals irradiated between 1948 and 1960, for control of tinea capitis, and two matched control groups (almost 16,000 people) are being retrospectively followed through national registries and medical records. The irradiated group has been found to have a significant excess of malignant and benign head and neck tumors, especially of the brain, parotid gland and the thyroid.

40. DERMATOGLYPHICS IN CHILDHOOD NEOPLASIA

Oorthuys, A. M., deVaan, G. A., Haanen, C. A., Katholieke Universiteit Nijmegen, Dept. of Human Genetics, *Erasmuslaan 4, Nijmegen, Netherlands*

OBJECTIVE: To characterize flexion creases and dermal configurations on palms and soles of patients with childhood neoplasia and of their first degree relatives.

APPROACH: Printing of palms and soles of pediatric patients with acute leukemia (AL) or with other neoplasms (malignant non-Hodgkin's lymphoma, Wilms' tumor, neuroblastoma, brain tumors, Hodgkin's disease) and their relatives.

PROGRESS: Material has been extensively enlarged; results remain consistent with earlier reports. Association between resistance to childhood neoplasia and early fetal development is feasible in so far that among young patients with AL a relation seems to exist between good prognosis and absence of variant dermatoglyphic features. Correlation of dermatoglyphic variance, defects of purine- and pyrimidine-metabolism, and HLA-typing, among patients with childhood neoplasia and their relatives, is the objective of further study.

41. EPIDEMIOLOGIC STUDY OF CHILDHOOD GLIOMAS

Nasca, P. C., State Bureau of Cancer Control, *Empire State Plaza, Tower Bldg. Rm. 565, Albany, New York*, 12237, United States of America

Our general objective is to determine, by means of an epidemiologic case-control study, the importance of various prenatal and childhood environmental exposures in the etiology of childhood gliomas. Special emphasis will be placed on prenatal and early childhood exposures to drugs, chemicals, radiation, tobacco, immunizations and animals. Selected birth characteristics including maternal age, parity and season of birth will also be examined.

Approximately 400 patients with childhood gliomas should be available for study. This sample size and a control-to-case ratio of 2:1 shall enable us to investigate the study variables with respect to the more common histologic subtypes. Demographic, pathologic and clinical data will be collected from the hospital records of each patient, utilizing a standardized form. Further data will be collected through telephone interviews with the mothers of each patient and control. These will be validated by comparing the mothers' responses with physician and hospital records.

42. CLASSIFICATION AND ETIOLOGY OF CHILDHOOD BRAIN TUMORS

Leviton, A., Gilles, F. H., Childrens Hospital Medical Center, *300 Longwood Ave., Boston, Massachusetts*, 02115, United States of America

This is a case-control epidemiologic study of brain tumors occurring in children. Cases are those children (ages 1 day to 16 years) with a brain tumor who reside in the catchment area of eight 'cancer' registries included in the SEER program. Three controls will be selected for each case by random digit dialing, matching for age (plus or minus 3 years), sex and race. A one and one-half hour long questionnaire will be administered to both parents by an interviewer in the parents' home. The questionnaire includes requests for information about demographic features, parents' exposure to possible teratogens and mutagens, familial history of tumor and birth defects, and exposure to infectious agents. During this project, questionnaires will be completed for approximately 400 cases and 1,200 controls. The sample size is expected to be sufficient for the study of the three most common (groups of) tumors (cerebellar gliomas, medulloblastomas, cerebral gliomas).

43. ETIOLOGY OF CHILDHOOD BRAIN TUMORS

Leviton, A., Austin, D. F., Child, M. A., Kolonel, L. N., Aschenbrenner, C., Albert, S., Key, C. R., Lyon, J. L., Weiss, N. S., Childrens Hospital Medical Center, Dept. of Neuroepidemiology, *300 Longwood Ave., Boston, Massachusetts*, 02115, United States of America

The purpose of this case-control study is to identify risk factors for the most common subtypes of the three most common site-specific brain tumors (cerebellar gliomas, medulloblastomas and supratentorial gliomas). Cases consist of all children with these types of brain tumors identified within the catchment areas of the eight participating SEER (Surveillance, Evaluation and End Results) Registries between 1/1/78 and 12/31/81. Three controls will be obtained for each case through Random Digit Dialing and will be matched for age (3 years), sex and race. Parents of cases and controls are asked by questionnaire to provide information on the numerous exposures that might influence the risk of brain tumors in children. The null hypothesis to be tested is that each variable has the same distribution among cases and controls.

44. PEDIATRIC NEUROEPIDEMIOLOGY

Schoenberg, B. S., Schoenberg, D. G., Mellinger, J. F., Gomez, M. R., Christine, B. W., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of

Neurological & Communicative Dis. & Stroke, Section on Neuroepidemiology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The project documented the frequency of primary intracranial neoplasms in the pediatric populations of Rochester, Minnesota, and the State of Connecticut. In addition, we investigated the magnitude and risk factors for cerebrovascular disease in infants and children in the Rochester, Minnesota population.

BIBLIOGRAPHIC REFERENCES: Schoenberg, B.S., Mellinger, J.F., and Schoenberg, D.G.: Cerebrovascular disease in infants and children: a study of incidence, clinical features, and survival. Neurology 28: 763-768, 1978.

45. FOLLOW-UP STUDY OF PATIENTS TREATED BY X-RAY EPILATION FOR TINEA CAPITIS

Albert, R. E., Shore, R. E., Pasternack, B. S., New York University, School of Medicine, Dept. of Environmental Medicine, *100 Washington Sq. E., New York, New York,* 10003, United States of America

Children treated for tinea capitis between 1940 and 1959 at a major New York City dermatology clinic have been followed to examine the tumourigenic effects of X-radiation to the head (300 - 400 R). Some 2,215 irradiated patients and 1,395 patients given medications for tinea capitis have been traced. The groups are very well matched on sex, race, age and socioeconomic status. A third survey of these groups (average interval since tinea treatment - 20 years) is in progress and is showing a continued excess of basal cell carcinomas, and brain, thyroid and parotid tumors in the irradiated group.

46. OHIO CENTRAL DATA BASE FOR CHILDHOOD CANCER

Newton, W. A., Dyment, P. G., Kastelic, J., Stout, R. A., Robinson, M., Gross, S., Lampkin, B., Ohio State University, Columbus Campus, Childrens Hospital, *561 S. 17th St., Livingston Pike, Columbus, Ohio,* 43205, United States of America

The objective is to provide baseline information on demographic patterns for cancer morbidity and mortality in Ohio children. This includes case-finding information regarding type of cancer by age, race, sex, place of residence, staging at initial diagnosis and institution of initial diagnosis. Abstracts are being prepared on hospital discharge of all newly diagnosed children with cancer beginning January 1975 from all the major treatment centers for children in Ohio. These abstracts utilize forms currently in use in a broader data collection program, the Children's Hospital Automated Medical Program (CHAMP), a medical research computer center located in Children's Hospital, Columbus, Ohio. In addition, an epidemiological questionnaire to be completed by parents is also being collected. This includes general questions on family history, including cancer in relatives and malformations, with additional specific information on patients with leukemia, brain tumors, Wilms' tumor and malignant lymphoma.

3. OCCUPATIONAL EPIDEMIOLOGICAL STUDIES OF NERVOUS SYSTEM TUMORS

47. MORTALITY STUDY OF UK ACRYLONITRILE WORKERS

Duncan, K. P., Werner, J. B., Jones, R. D., Health & Safety Center, *97 Horseferry Rd., London SW1P 2DY, England, United Kingdom*

The study takes the form of an examination of the mortality pattern of 1,300 acrylonitrile polymerization process workers who were first exposed to acrylonitrile in 1968 or earlier in six factories in the UK. Data on job histories and exposure levels will be used to allocate workers to defined exposure groups. Mortality from all causes of death, together with selected causes including cancer of lung, brain and large intestine, will be considered.

48. CANCER INCIDENCE AMONG EMPLOYEES IN THE CHEMICAL INDUSTRY

Ahlbom, A. O., Norell, S. E., Olin, R. G., Huddinge Sjukhus, *S14186 Huddinge, Sweden*

The aim of the study is to assess the association between chemical exposure at work and leukemia and brain tumors. The study is based on the Swedish Cancer Registry. This was linked to the census of 1960 which included information about occupation. The material consists of cases reported to the Registry during the period 1961 to 1973.

49. CASE-CONTROL STUDY ON BRAIN TUMORS AND OCCUPATION

Musicco, M., Filippini, G., Morello, G., Berrino, F., C. Besta Neurological Inst., Dept. of Environmental & Occupational Neuropathology, *Via Celoria n.11, 20133 Milan, Italy*

The etiology of brain cancer in humans is mostly unknown; a number of chemicals, however, have been shown to induce tumors of the nervous system in animals (mainly nitrosureas), and a few are suspected of inducing human cancer. In this study the exposures of cases and controls are assessed from data on occupational history and specific chemical exposures, obtained by interview. Following preliminary data showing an excess of farmers among cases, specific questions on herbicides and pesticides have been added. X-ray exposure and smoking and drinking habits are also included in the questionnaire. Cases are all patients with a definite diagnosis of glioma or meningioma admitted to this Institute since 1978 (about 100 cases of glioma and 100 cases of meningioma each year). Two controls for each case are matched by sex, age, date of admission and residence.

50. PROSPECTIVE AND RETROSPECTIVE COHORT STUDIES OF MORTALITY AND MORBIDITY AMONG SWEDISH CHEMISTRY GRADUATES

Olin, R. G., Unander, B. D., Alvarsson, B., Kungliga Tekniske Hogskolan, Dept. of Safety & Health, *S10044 Stockholm 70, Sweden*

The causes of death among chemists who belonged to a cohort of men who graduated from the School of Chemical Engineering at the Royal Institute of Technology in Stockholm during the years 1930-1960 were studied. The group was followed until the end of 1977. Results to date show a significant increase in cancers, particularly malignant lymphomas and brain and prostate neoplasms in comparison with the causes of death in the general population, as well as in a similar cohort of architects educated at the same school during the same period of time. The study is at present being continued prospectively. The surviving male chemists have been sent a questionnaire concerning exposures and health effects; the participation rate is about 85%.

51. EPIDEMIOLOGIC STUDY OF WORKERS IN PETROLEUM REFINERIES AND PETROCHEMICAL PLANTS

Waxweiler, R., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance, Hazard Evaluations & Field Studies, *4676 Columbia Pkwy., Cincinnati, Ohio,* 45226, United States of America

An NCI study examined the cause-specific mortality experience of workers who were active members of Oil, Chemical and Atomic Workers International Union (OCAW) Locals in Texas at the time of death. Proportional mortality ratios (PMR's) for stomach and brain cancers, for leukemia, and for multiple myeloma were elevated among white males who worked in petroleum refineries and petrochemical plants and were Union members for at least 10 years. In particular, increased relative frequencies of stomach and brain cancer deaths were found among white male members who were employed at a refinery in Port Arthur (Plant A). In addition, PMR's were elevated for lung cancer among non-whites and stomach cancer in both racial groups employed at another refinery in Port Arthur (Plant B).

Observed numbers of deaths from leukemia, and from cancers of the kidney and skin were greater than expected among white males at Plant B, but observed numbers were very small.

A third refinery in Beaumont, Texas, was found to have excessive frequencies of lung and brain cancers among workers. A case-control study will be done as a timely second step to examine excess deaths from certain cancers in greater detail.

Detailed work histories will be obtained from each case and control to determine length of employment in these plants, specific job(s) held, work location(s) within the plants, and any other employment or exposure information that is available.

The case-control study may be followed by a more definitive cohort study.

52. MEDICAL, MORTALITY AND INDUSTRIAL HYGIENE STUDY OF WORKERS

Fajen, J., Egan, B., Smith, B., Fine, D., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance, Hazard Evaluations & Field Studies, *4676 Columbia Pkwy., Cincinnati, Ohio*, 45226, United States of America

Dimethylnitrosamine is labelled as a carcinogen by the Occupational Safety and Health Administration and therefore in the few areas where it is used or manufactured, appropriate precautions are taken. It has only recently been discovered through the development of new analytical techniques that dimethylnitrosamines and other nitroso compounds may form in the ambient atmosphere or even in vivo if sufficient concentrations of nitroso compound precursors (secondary amines, amides, etc.) and nitrates, nitrites, or other nitrogenous compounds exist under appropriate conditions.

Twenty-one industrial plants have been investigated for any possible nitrosamine contamination. The industries visited thus far have been the automobile, azo dye, small machine shops, fish and fish meal, rubber and leather. To date N-nitrosodiethanolamine, N-nitrosodimethylamine, N-nitrosomorpholine, and N-nitrosodiphenylamines have been found in the air of several plants. N-nitrosodimethylamine, a regulated carcinogen, was found as high as 50 micrograms/cubic meter in the leather industry. Efforts are being made to determine the nitrosating agent, the extent of human exposure, and methods of controlling the exposure to N-nitrosodimethylamine.

Detailed industrial hygiene and biological sampling will be conducted in the rubber and leather industries as well as newly identified industries to determine the level and extent of nitrosamine exposure and to identify conditions necessary for spontaneous formation of these compounds.

An ophthalmologic survey was undertaken at the E.I. duPont de Nemours and Company, Belle, West Virginia plant, where an excess of eye tumors has been noted and where nitrosamines have been found in the ambient environment. Data analysis will proceed in 1979, and follow-up studies of participants in that survey will be conducted.

53. MORTALITY STUDY OF INCREASED BRAIN TUMOR INCIDENCE IN TEXAS CITY, TEXAS

Leffingwell, S., Waxweiler, R., Ludwig, H., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance, Hazard Evaluations & Field Studies, *4676 Columbia Pkwy., Cincinnati, Ohio*, 45226, United States of America

In response to a complaint of alleged excessive brain tumors among employees at the Union Carbide Company of Texas City, Texas, the Houston South Area Office of OSHA launched an investigation in November of 1978. After a careful inquiry, OSHA requested technical assistance from NIOSH in evaluating the problem.

Preliminary data, supplied with aid of the company, local unions, and local physicians and surgeons, show ten cases in which death certificates indicated brain tumor as cause of death. One additional living employee was found to have a brain tumor. The cell types were predominantly glioma or glioblastoma multiforme with one 'meningioma, angioblastic (sarcoma)'. The workforce at the plant presently numbers 2500. Between 7.5 and 10 thousand workers have worked in the plant between 1941, when the plant began operation, and the present.

Present plans call for additional case-finding, case confirmation by pathological review of tissues, a proportional mortality study (as a check of methodology which may be useful elsewhere), a case-control study of brain tumor deaths, and a cohort mortality study. Coding of company records is being completed and the case-control study will begin soon.

54. CONTINUATION OF DEMONSTRATION CANCER CONTROL PROGRAM IN INDUSTRY

Tamburro, C. H., Greenberg, R. A., University of Louisville, School of Medicine, Dept. of Community Health, *101 W. Chestnut, Louisville, Kentucky*, 40202, United States of America

OBJECTIVE: To assess data gathered from an NCI Cancer Control Project Contract NO1-CN-55212: 'Implementation and Assessment of a Demonstration Cancer Control Detection and Prevention Program in a Cohort of Industrial Workers.'

APPROACH: A cancer control demonstration project was implemented in Louisville after the discovery of hepatic angiosarcoma, a rare cancer, among polyvinyl chloride workers. This cohort is still undergoing medical surveillance and data is being stored and analyzed. This ongoing project is currently assessing the data gathered from the medical surveillance of these workers. The data base provides for evaluation of ill employees individually, provides monitoring of screening compliance, provides subcohorts for special studies, provides secondary non-invasive epidemiological research studies and finally a base for design and sample size consideration for future investigative projects.

PROGRESS: The sensitivity and specificity of federally required screening tests as well as others is being determined. Twenty-two chemicals besides vinyl chloride are undergoing scrutiny as to their potential for causing disease. High and low risk subgroups have also been identified as to non-malignant hepatic and splenic disease. Four major primary sites of cancer development in the chemical (industrial) environment are under study in addition to hepatic angiosarcoma: A) Lung cancer, particular large cell carcinoma, B) bilateral pleural thickening and possible relationship to inadvertent asbestos exposure, C) colon cancer and acrylonitrile exposure and D) brain cancer and vinyl chloride exposure.

55. CASE-CONTROL STUDY USING SSA IDENTIFICATIONS

Halperin, W., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance, Hazard Evaluations & Field Studies, *4676 Columbia Pkwy., Cincinnati, Ohio*, 45226, United States of America

This study presents a new methodologic approach to linking disease in a geographic area to an occupational etiology. Examples include bladder cancer in New Jersey and leukemia or brain tumors in east Texas. The Social Security Administration records the employer, for each contributor, for each quarter. For any individual, reconstruction of a continuous employer history is feasible.

We propose first to ascertain cases of a particular disease, such as bladder cancer in New Jersey, and controls, consisting of New Jersey citizens of similar age, race, and sex with other causes of death. SSA would then provide continuous work histories for cases and controls. Specific employers or categories of employers represented in excess among cases as compared to controls may then be identified. These industries or employers would then be subject to in-depth cohort mortality studies. The cohort mortality study may identify new etiologies for the disease in question or identify continuing exposures to known carcinogens.

56. WORKSHOP ON BRAIN TUMORS IN THE CHEMICAL INDUSTRY

Selikoff, I. J., New York Academy of Sciences, Environmental Sciences Laboratory, *2 E. 63rd St., New York, New York*, 10021, United States of America

In November of 1978 the Occupational Safety and Health Administration received a complaint from an employee who stated that he was suffering from a brain tumor and knew of others with the same problem. This spurred a series of epidemiological investigations to determine whether this was a random occurrence or whether these tumors might be related to worker exposure to toxic substances at the plant. At the one facility in question, 18 brain cancer deaths have occurred to date and 25 other cases have been indentified at another plant; similar cases are being reported in petrochemical facilities in other parts of the world. It is our intention to bring together scientists from government, industry, academic institutions and labor to review the current state of the knowledge concerning occupational and environmental etiology of brain tumors. The consideration of currently available data will allow dissemination of state-of-the-art information to researchers throughout the world. It will further serve to assist in evaluation of etiological factors, of particular significance now as measures are considered for control of the problem.

57. MULTIPLE MYELOMA AND BRAIN TUMORS IN PHYSICIANS

Matanoski, G. M., Diamond, E. L., Elliott, E. A., Johns Hopkins University, School of Hygiene & Public Health, Dept. of Epidemiology, *615 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

Studying the etiology of these two cancers and their correlation in physicians, a relatively homogenous population which is unique in having a high probability of exposure to repeated low-dose radiation and infections and good medical histories. The design is a case-control study with controls comparable by age, race, sex, and year of death selected from total physician deaths over fifteen years. An equal number of living controls will also be selected. Risk factors identified in previous studies will be examined by telephone interviews with next-of-kin, physicians associates, or physicians if a case-control. There will be special emphasis on determining the exact number of years of exposure to radiation and the estimated dose levels by year or dosimetry data. Questions concerning patient contacts, personal characteristics and medical history will be included. Information on residence, type of training and speciality are available from AMA records. A multi-variate analysis of data will be used to determine association of each of these cancers with radiation, infection, or other factors.

58. URANIUM MINER AND MILL STUDIES - MINING

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During the period 1950-1975, NIOSH collected data on about 20,000 uranium miners and mill workers. Further study, including follow-up through 1977, and analysis of these data are expected to improve the exposure-response curve for lung cancer, to provide data on hair root changes, to determine if an altered sex ratio of offspring for uranium miners exists, and to determine if there is an excess of leukemia, lymphoma, cancer of the brain, pancreas, or bone in uranium workers.

4. OTHER EPIDEMIOLOGICAL STUDIES OF NERVOUS SYSTEM TUMORS

59. EPIDEMIOLOGICAL STUDY OF CEREBRAL TUMORS IN ADULTS

Burch, J. D., Howe, G. R., Miller, A. B., National Cancer Inst. of Canada, Epidemiology Unit, Dept. of Preventive Medicine & Biostatistics, *12 Queen's Pk. Cres. W., c/o University of Toronto, Toronto, Ontario*, M5S 1A8, Canada

Little is known of the etiology of cerebral tumors in humans, although associations have been suggested as a result of incidence, mortality, and animal studies. These include urban/rural differences, occupation with specific reference to farming, the rubber industry, and exposure to vinyl chloride, familial associations, ionizing radiation, brain trauma, and specific chemical carcinogens. The current study will re-investigate these associations and explore other possible relevant factors. The study will test the hypotheses that cerebral tumors are associated with specific occupations (farming, rubber industry, plastics industry), family history of brain tumor, and exposure to nitrosamines, especially through drinking water and preservative-containing meats and meat products. A number of questions will also be asked of respondents relating to additional environmental factors (e.g., smoking, alcohol consumption, etc.). Data on past events and exposures will be obtained from cases and hospital controls through personal interviews in the respondents' homes using proxy respondents for dead or incapacitated cases and appropriate proxy controls. Case and control acquisition is restricted to the metropolitan Toronto and fringe areas and will include incident cases with defined histology for the four-year period 1 January 1977 to 31 December 1980.

60. GEOGRAPHICAL DISTRIBUTION OF MALIGNANT NEOPLASMS OF THE BRAIN IN CANADA

Neutel, I. C., University of Ottawa, Faculty of Medicine, Dept. of Epidemiology & Community Medicine, *1145 Carling Ave., Lady Grey Bldg., Ottawa, Ontario*, K1Z 7K4, Canada

Malignant neoplasms of the brain have received very little epidemiological attention in spite of the low survival rate and their occurrence in childhood or early adulthood. A few studies dealt with their general distribution in the United States and Denmark and only one studied the geographical distribution in relation to environmental factors. This project will study the geographical distribution of brain tumours across Canada using both mortality and incidence data by calculating age-adjusted mortality and incidence rates for census divisions. These rates can be related to various environmental variables known for the census divisions. In addition, this study will investigate the occupations recorded for a selected group of brain tumour deaths and compare them with those of a matched group of controls. Of special interest will be occupations likely to involve chemical or radiation exposure. Prenatal radiation has been shown to increase the risk of childhood cancer of the central nervous system, and the same situation may apply to adult brain cancer.

61. NEURAL NEOPLASMS IN THAILAND

Shuangshoti, S., Panyathanya, R. I., Netsky, M. G., Chulalongkorn University, School of Medicine, Dept. of Pathology, *Phya Thai Rd., Bangkok, Thailand*

OBJECTIVES: (1) To study relative proportions of type, age, sex and location of all neoplasms of the nervous system in Thailand, (2) to study individual types of neural neoplasms, (3) to study highly interesting or unique cases of neural neoplasms and report them separately.

SCOPE: Review of surgical specimens and postmortem materials from two large general university hospitals in Bangkok: Chulalongkorn Hospital and Siriraj Hospital. Materials from other hospitals in Bangkok and from the rest of the country, sent in for consultation, are also included.

62. PRIMARY INTRACRANIAL TUMORS IN TRIESTE

Bianchi, C., Grandi, G., Berti, N., Di Bonito, L., Universita degli Studi di Trieste, Fac. di Medicina, Inst. of Pathological Anatomy & Histology, *via Stuparich 1, Trieste, Italy*

Cases in which a diagnosis of primary intracranial tumor is made at biopsy or autopsy in the Province of Trieste will be collected. Detailed personal and medical data will be obtained for each case from the patient or relatives by personal interview. In this, attention will be focused on the following points: diet, tobacco and alcohol habits, residence during different periods of life, occupational history, contacts with animals, vaccinations, viral diseases, diagnostic and therapeutic radiations, drug use, etc.

63. GENETICS OF RETINOBLASTOMA

Briard, M. L., Bonaiti, C. G., Feingold, J., Frezal, J., Inst. National de la Sante et de la Recherche Medicale, Unite de Recherche Genetique Medicale, *149 rue de Sevres, c/o Hopital Enfants Malades, 75015 Paris, France*

It is now recognized that bilateral retinoblastoma is a condition with dominant autosomal transmission and complete penetrance, but the mode of transmission of the unilateral form is not yet clear. This study aims at monitoring 600 families that have been registered through at least one proband and examining the offspring of patients during the next generation.

64. EPIDEMIOLOGY OF INTRACRANIAL NEOPLASMS

Preston Martin, S., Henderson, B. E., Gardner, M. B., Mack, T. M., University of Southern California, School of Medicine, Dept. of Pathology, *2025 Zonal Ave., Los Angeles, California*, 90033, United States of America

A large case-control study of two subgroups of patients with primary intracranial neoplasms is to be performed in Los Angeles County: intracranial tumors of all histological types first occurring in children and young people under age 25 from 1972 to 1977; and incident intracranial meningiomas in men from 1972 to 1975. The study has been designed to test the hypothesis that previous radiation exposure is causally related to the development of these tumors, to test previously suggested causal associations, to discover new associations and to delineate the characteristics of the high risk groups. Cases with specified intracranial tumours will be identified by the Los Angeles County-University of Southern California Cancer Surveillance Program. Questionnaires have been designed to obtain data on radiation exposure, occupation, consumption of tobacco and drugs, medical history, and family history of tumors. Standard matched pair analyses of multiple variables will be performed.

65. THE EPIDEMIOLOGY OF INTRACRANIAL NEOPLASMS

Schoenberg, B. S., Christine, B. W., Whisnant, J. P., Campbell, R. J., Kudrjavcev, T., Mahalak, L., Heck, A., Simon, R., Jordan, B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Neurological & Communicative Dis. & Stroke, Section on Neuroepidemiology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The Section has conducted extensive investigations into the descriptive epidemiology of primary intracranial neoplasms using data derived from population-based registries worldwide. Analytic studies were carried out to investigate the relationship between intracranial neoplasms and tumors occurring at other sites. These studies included careful review of tumor nomenclature, disease definitions, and survey strategies.

BIBLIOGRAPHIC REFERENCES: Schoenberg, B.S., Christine, B.W., and Whisnant, J.P.: The resolution of discrepancies in the reported incidence of primary brain tumors. Neurology 28: 817-823, 1978.

66. EPIDEMIOLOGY OF BRAIN TUMORS

Hochberg, F. H., Cole, P., Massachusetts General Hospital, Neurology Service, *32 Fruit St., Boston, Massachusetts,* 02114, United States of America

This is a case-control study involving 230 patients and 230 'best friend' controls designed to elucidate risk factors in the development of primary glial tumors of the brain. The control, who is provided by the patient, is a person of the same sex, within 5 years of age, living within 100 miles and not a blood relative. Both patients and controls are given a 45 page questionnaire to complete. Telephone followups are performed to further clarify responses. Areas covered by the questionnaire include family history, medical background, environmental and occupational exposures.

67. EPIDEMIOLOGIC STUDY OF BRAIN TUMORS IN A POPULATION EXPOSED TO CHLORINATION BY-PRODUCTS

Greathouse, D. G., U.S. Environmental Protection Agency, Office of Research & Development, Health Effects Research Lab., *5555 Ridge Ave., Cincinnati, Ohio,* 45268, United States of America

OBJECTIVE: Assist the prinicipal investigator of a cooperative agreement in the development of a study protocol to investigate the role of chlorination by-products in the development of brain tumors.

APPROACH: Participate in planning and review meetings.

68. PRIMARY INTRACRANIAL NEOPLASMS IN ROCHESTER, MINNESOTA, 1935-1978

Annegers, J. F., Schoenberg, B. S., Kurland, L. T., Okazaki, H., Mayo Clinic, Dept. of Medical Statistics & Epidemiology, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

This study is expected to provide data on the incidence of intracranial neoplasms in the population of Olmsted County from 1935 through 1977. The incidence rates will be described by histological type, age, sex and time period. In addition of symptomatic tumors discovered during a patient's lifetime, the age- and sex-specific prevalence rates of brain tumors found at autopsy will be determined. In addition to providing incidence rates of brain tumors, this study will address the implications of cases diagnosed incidentally at autopsy.

69. PRELIMINARY STUDY ON THE EPIDEMIOLOGY OF MENINGIOMAS

Lin, R. S., Bracket, C. E., Morantz, R. A., University of Maryland, Baltimore Professional Schools, School of Medicine, Dept. of Epidemiology & Preventive Medicine, *655 W. Baltimore St., Baltimore, Maryland,* 21201, United States of America

The objective is to identify risk factors for meningioma with special emphasis on the use of hair-dyes. The possible etiological factors of trauma, irradiation, infection, sex hormones and drugs, hereditary factors and other environmental exposures will also be assessed. A retrospective case-control method by means of mailed questionnaires is used, cases being meningioma patients diagnosed at the University of Kansas Medical Center. For each female case, three control patients without cancer, matched for race and year of birth, are selected from the name-roster at the Breast Cancer Detection Center of the University of Kansas Medical Center. Preliminary analyses from the collected questionnaires of 41 female cases with meningioma and 86 controls show that meningioma cases reported themselves exposed to hair-dyes less frequently than the controls. However, meningioma cases exhibit a significantly higher body weight and body mass index (RMT) at age 40 than the controls. In addition, more cases than controls come from large families and suffer from thyroid diseases. The epidemiological findings as well as the well known clinical evidence of a femele preponderance in meningiomas seem to suggest that hormonal factors may play an important role in the pathogenesis. Further exploration of the association with hormonal imbalance or obesity is planned.

70. HEAD TRAUMA AND SUBSEQUENT BRAIN TUMORS

Annegers, J. F., Laws, E. R., Kurland, L. T., Mayo Clinic, Dept. of Epidemiology, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

A total of 2,953 patients with significant head injuries are being followed to determine the occurrence of intracranial neoplasms after head trauma. The observed number will be compared to the expected number based on local incidence rates of intracranial neoplasms.

71. INCIDENCE, PREVALENCE AND COSTS OF INTRACRANIAL NEOPLASMS

Eldred, C., Westat Inc., *11600 Nebel St., Rockville, Maryland,* 20852, United States of America

OBJECTIVES: Develop methods, procedures, definitions, questionnaires, and abstraction forms for a survey of intracranial neoplasms. Develop, conduct, and analyze a pilot study to conduct these protocols. Design, conduct, and analyze a national survey to provide estimates of the incidence and prevalence of intracranial neoplasms by demographic subgroups of the U.S. population. Obtain estimates of the medical and other costs of intracranial neoplasms.

72. REPORTED INCIDENCE OF PRIMARY BRAIN TUMORS (HUMAN)

Schoenberg, B., Mayo Foundation, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

This is part of a broader project. A summary of this subproject is not available.

73. FAMILY WITH NEUROFIBROMATOSIS AND MALIGNANT PHEOCHROMOCYTOMA

Hakami, N., University of Missouri, Columbia Campus, School of Medicine, Dept. of Child Health, *M228 Medical Sciences, Columbia, Missouri,* 65201, United States of America

This is part of a broader project. A summary of this subproject is not available.

II. DIAGNOSIS OF NERVOUS SYSTEM TUMORS

A. IMMUNOLOGICAL METHODS

74. TUMOR-SPECIFIC MEMBRANE ANTIGENS IN HUMAN GLIOMAS

Birkmayer, G. D., Ludwig Maximilians Universitat Munchen, Dept. of Cell Biology, *Goethestr. 33, D8000 Munich 2, Bayern, Federal Republic of Germany*

OBJECTIVE: Isolation and characterization of tumor-specific membrane antigens in human gliomas in order to establish biochemical and immunological assays for diagnosis of human brain tumors.

APPROACH: A tumor-specific antiserum raised against cell-membrane components is used as a tool for detection of tumor-specific membrane antigens in human gliomas. By the use of the nonionic detergent, Triton X-100 is solubilized and purified by preparative polyacrylamide electrophoresis. Antisera against these antigens are raised in rabbits and in sheep and absorbed to various components extracted from membranes of normal human brain and lymphocytes.

PROGRESS: By a rabbit antiserum against the Triton extract from membranes of human gliomas, 2 membrane associated

proteins have been detected which were absent in normal brain tissue, normal glia cells grown in vitro and normal lymphocytes. By immunodiffussion, counter-current electrophoresis and immunofuorescence, these antigens have been detected in all the 25 gliomas tested so far.

Electrophoretic analysis of the immunoprecipitate revealed in addition to the two bands of the immunoglobulin, one band in the molecular weight region of 70,000 daltons and the other in the region of 12,000 daltons molecular weight. Twenty (85%) of 25 patients suffering from gliomas have precipitating antibodies in their sera, which cross-react with the Triton-extract from the membrane of human gliomas.

75. DEVELOPMENT OF A RADIOIMMUNOASSAY FOR AN ONCOFETAL ANTIGEN OF POTENTIAL USE IN THE DIAGNOSIS OF HUMAN BRAIN TUMORS

Brock, D. J., University of Edinburgh, Dept. of Human Genetics, *Mayfield Rd., Edinburgh EH9 3JR, Scotland, United Kingdom*

OBJECTIVE: We aim to develop a radioimmunoassay for a new oncofetal antigen identified in both fetal brain and human brain tumors.

APPROACH: Neonatally tolerized rabbits challenged with preparations of human fetal brain tissue have responded by producing antisera, which after absorption with adult brain tissue, recognize an antigen present only in fetal brain and human brain tumors. The antiserum will be used to prepare affinity columns with the purpose of isolating the antigen. This in turn will allow the development of a radioimmunoassay. The RIA will be used to put the potential of the antigen in early diagnosis of human brain tumors.

76. IMMUNE RESPONSE OF CHILDREN TO NEUROBLASTOMA

Seeger, R., University of California, Los Angeles Campus, School of Medicine, Dept. of Pediatrics, *2025 Zonal Ave., Los Angeles, California*, 90024, United States of America

There is a well recognized need to improve therapy for two-thirds of children with neuroblastoma. This investigation will generate monoclonal antibodies to neuroblastoma associated fetal and/or neural cell surface antigens (CSA), to neurotransmitter-synthesizing enzymes, and to neuroblastoma produced auto-stimulatory growth factors. Our approach is to develop a panel of antibodies because the simultaneous use of two or more antibodies is likely to improve many aspects of immunodiagnosis and immunotherapy; multiple antibodies will be particularly useful in dealing with the problem of tumor heterogeneity. Monoclonal antibodies will be used for clinical diagnostic and preclinical therapeutic investigations. Clinical diagnostic studies will determine (1) the prognostic value of antigenic phenotyping at diagnosis and (2) the ability of immunoenzyme and immunofluorescent analysis of bone marrow and radioimmunoassay of serum to accurately assess tumor burden 25 weeks after diagnosis. Preclinical antibody therapy studies will use neuroblastoma cell lines growing in vitro and in athymic nude mice to investigate (1) antibodies that cooperate with human complement and effector cells to mediate complement dependent or antibody dependent cellular cytotoxicity and (2) antibodies that affect proliferation and survival of neuroblastoma cells independent of complement and effector cells. Having identified antibodies that are effective alone, combinations will be tested to identify those that are additive or synergistic. If successful, these preclinical studies could be the basis for designing clinical antibody therapy for neuroblastoma. These investigations of clinical immunodiagnosis and preclinical antibody therapy should significantly contribute to our long-term aim of achieving optimal therapy for children with neuroblastoma.

77. EYE TUMORS - IN VITRO IMMUNODIAGNOSIS AND MONITORING

Char, D. H., University of California, San Francisco Campus, School of Medicine, Dept. of Ophthalmology, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

Immunologic mechanisms may be important in ocular tumors. We have previously demonstrated cellular reactivity towards melanoma-associated antigens in patients with choroidal melanoma. Studies are continued to determine whether the use of highly purified melanoma-associated antigens in in vitro assays will allow us to better predict which patients with choroidal melanoma are at greater risk of developing metastatic disease.

In retinoblastoma we have demonstrated the existence of immune complexes. Current work is being performed to determine the nature of the antigen and antibody moieties which make up the immune complexes. Serial studies are being performed on retinoblastoma patients to determine whether immune complex levels will correlate with disease status and prognosis.

In patients with orbital pseudotumors and lymphomas, the histologic diagnosis is inaccurate in up to 50 percent of cases. Using lymphocyte surface markers studies, we may be better able to differentiate these two types of orbital mass lesions which have a markedly different prognosis. Studies on the specificity, sensitivity, and reproducibility of immunologic lymphocyte surface marker studies in patients with ocular disease are continuing.

78. CELL SURFACE ANTIGENS OF CHILDHOOD BRAIN TUMORS

Zelter, P. M., University of Texas, Health Science Center at San Antonio, Medical School, Dept. of Pediatrics, *7703 Floyd Curl Dr., San Antonio, Texas*, 78284, United States of America

OBJECTIVE: The overall objective of this project is to develop specific and sensitive serologic assays for brain tumor antigens which may provide improvements over techniques currently available for diagnosis, classification and monitoring of these neoplasms in children.

APPROACH: CSA (tumor related, fetal, differentiation type) of these brain tumors will be identified and later characterized, using serologic and molecular techniques which were developed in this laboratory. Next, three types of investigations using these CSA will be implemented: 1) determination of tumor CSA phenotype, 2) quantitation of soluble CSA in cerebrospinal fluid, serum, and/or urine by radioimmunoassay, and 3) determination of human anti-CSA antibody responses. The value of these tests for diagnosis, prognosis, and therapeutic assessment will be determined by correlation with clinical parameters.

79. VASOACTIVE INTESTINAL AND LUNG POLYPEPTIDES - TUMOR

Said, S. I., Mutt, V., Bodanszky, M., University of Texas, Health Science Center at Dallas, Southwestern Medical School, Dept. of Internal Medicine, *5323 Harry Hines Blvd., Dallas, Texas*, 75230, United States of America

This proposal deals with the relationship of three newly identified polypeptides, vasoactive intestinal peptide (VIP) and two vasoactive lung peptides, to a number of malignant tumors. Using a sensitive and specific radioimmunoassay (RIA), VIP has been shown to occur normally in the gastrointestinal and nervous systems, and in elevated concentrations in these tumors: pancreatic (islet-cell) adenomas, pheochromocytoma, neuroblastoma, medullary thyroid carcinoma, and bronchogenic carcinoma. The clinical syndrome in common among these tumors was that of watery diarrhea (pancreatic cholera), accompanied in some instances by hyperglycemia, hypercalcemia, hypokalemia and episodic flushing. One vasoactive lung peptide, extractable from normal lung, is related to VIP, cross-reacting with it in the RIA. The other vasoactive lung peptide is spasmogenic and does not cross-react with VIP. Determination of the prevalence of secretion of these peptides in the presence of malignant disease may provide useful clues to the early diagnosis, better management and, possibly also, the pathogenesis of these lesions.

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80. VASOPRESSIN IN ADULT AND FETAL BRAIN AND TUMORS (HUMAN)

George, J. M., Ohio State University, Columbus Campus, School of Medicine, Dept. of Medicine, *370 W. 9th Ave., Columbus, Ohio*, 43212, United States of America

This is part of a broader project. A summary of this subproject is not available.

B. BIOCHEMICAL METHODS

81. PROPERTIES OF REGULATORY ENZYMES OF GLYCOLYSIS AND PURINE METABOLISM IN NEOPLASIA

Staal, G. E., Rijksen, G., vanVeelen, C. W., Kraayenhagen, R. J., State University Hospital, Unit of Medical Enzymology, *Catharijnesingel 101, 3511GV Utrecht, Netherlands*

Pyruvate kinase isozymes were studied in normal brain tissue and in meningiomas and malignant gliomas. Pyruvate kinase from meningiomas and malignant gliomas is strongly inhibited by alanine, whereas pyruvate kinase from normal brain is not inhibited. Therefore the determination of the alanine inhibition of pyruvate kinase may be a diagnostic tool in surgery for gliomas (Thesis by C. van Veelen, in press). In view of the results obtained with the enzyme pyruvate kinase, two other enzymes will be studied in brain tumors: hexokinase and phosphofructokinase. The enzymes will be characterized with respect to isozyme shift, kinetics and electrophoresis. Furthermore, the possible correlation between malignancy and isozyme composition will be studied. The investigations will be carried out in collaboration with the Department of Neurosurgery of the Academic Hospital in Utrecht, The Netherlands.

Hexokinase was studied in cultured lymphoblasts (CEM-cell line). Besides hexokinase type I, type II is present too; normal lymphocytes have only type I. Furthermore, hexokinase was not influenced by inorganic phosphate. This means that some regulatory properties are absent. Methotrexate showed little or no influence on the hexokinase activity. Other regulatory enzymes such as phosphofructokinase, pyruvate kinase and some regulatory enzymes of purine metabolism will be studied with respect to isozyme composition and properties and the influence of methotrexate.

82. DETECTION OF OCULAR TUMORS

Tapper, D., Zetter, B. R., Harvard University, Boston Campus, Childrens Hospital Medical Center, *300 Longwood Ave., Boston, Massachusetts*, 02115, United States of America

Solid tumors exhibit the property of angiogenesis, i.e. the capacity to induce the proliferation of new capillary blood vessels from the host. The continued growth of a tumor appears to be bound to angiogenesis. It has been shown that angiogenesis is an early marker of pre-neoplastic lesions. Furthermore, we have shown that the angiogenesis capacity of the aqueous humor of human eyes could be used as a diagnostic marker for malignant eye lesions.

We now propose to determine if the aqueous humor of patients with eye tumors will stimulate capillary endothelial migration in a significantly greater fashion than the aqueous humor of patients with non-malignant conditions. Such an assay might be used as a quantitative method for ocular malignancies. This will be done using capillary endothelial cells which we have recently isolated, cloned, and maintained in culture. The basis for this assay is that these capillary cells, unlike endothelial cells from the aorta or umbilical veins, will only grow and migrate in the presence of tumor-conditioned medium. Preliminary evidence has been obtained which shows that substances causing angiogenesis in vivo cause migration of capillary endothelial cells, but not other cell lines, in vitro.

We plan to measure the migration of capillary endothelial cells by means of a phagokinetic track assay of aqueous humor obtained from patients undergoing ophthalmologic surgery for both malignant and non-malignant eye lesions. We propose to test whether malignant lesions of the eye can be distinguished from non-malignant lesions based on the ability of the aqueous humor to stimulate the migration of capillary endothelial cells. In addition, we plan to determine to what extent capillary endothelial cell migration in vitro correlates with angiogenesis in vivo.

Finally, we will investigate the possibility of using this diagnostic method to detect patients at high risk of developing malignant eye lesions such as siblings of patients with retinoblastoma.

83. CLINICAL, GENETIC, PATHOPHYSIOLOGIC STUDY OF HEREDITARY NERVOUS SYSTEM TUMORS

Eldridge, R., Kanter, W., Todaro, G., Fabricant, R., Koerber, T., Smith, B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Neurological & Communicative Dis. & Stroke, Neurogenetics Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

In this project we seek to define and classify hereditary tumors of the nervous system in addition to the eight such diseases already recognized; to add to the clinical description and natural history of these diseases; to suggest methods for early diagnosis; to evaluate present modes of treatment; and to develop methods for preclinical detection and screening.

We have documented a central form of neurofibromatosis. In cooperation with several collaborators, we have demonstrated distinct alteration in nerve growth factor in blood in affected individuals and approximately half of those at risk.

BIBLIOGRAPHIC REFERENCES: Kanter, W., and Eldridge, R.: Maternal effect in central neurofibromatosis. The Lancet, 2: 8095, 903, 1978. Fabricant, R.N., Todaro, G.J., and Eldridge, R.: Increased levels of a nerve-growth factor cross-reacting protein in 'central' neurofibromatosis. The Lancet 1: 8106, 4-7, 1979.

84. HUMAN CSF PROTEINS IN TRAUMA AND DEMYELINATING DISORDERS

Sprinkle, T. J., Smith, D. B., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *Wrightsboro Rd., Augusta, Georgia*, 30904, United States of America

The general aims of the proposed research are: To continue and expand our initial studies on the correlation of myelin basic protein and CNP activity levels in human cerebrospinal fluid with the activity of demyelinating diseases.

To determine the levels of CNP and myelin basic protein in the cerebrospinal fluid of patients with traumatic brain and spinal cord lesions, stroke, lacunar state, certain dementias, brain tumors, and other neurological conditions that may involve destruction of myelin.

To determine the predictive value of CNP and myelin basic protein measurements on the clinical course of patients with demyelinating diseases or other neurologic disorders in which myelin is affected.

To compliment the clinical studies with further biochemical studies on the CNP enzyme, specifically, to isolate and characterize human myelin CNP and to develop specific antibodies to this protein.

To utilize the antibodies produced to establish in man the reactivity between the human peripheral nervous system enzyme and that from the human red blood cell.

C. RADIOLOGICAL METHODS

85. NON-CONTACT RADIONUCLIDE DETECTION OF OCULAR MELANOMA

Oosterhuis, J. A., van Langevelde, A., Pauwels, E. K., Kaspersen, F. M., Academisch Ziekenhuis Leyden, Dept. of Ophthalmology, *Rijnsburgerweg 10, 2333 AA Leyden, Netherlands*

OBJECTIVE: In order to be able to use the gamma camera as a non-invasive technique for the detection of intraocular melanoma, research is directed towards the availability of compounds which can be labeled with a gamma emitting radionuclide and which accumulate in eye melanoma.

APPROACH: In an animal model (Syrian golden hamster) with a transplantable Greene melanoma, the biodistribution of quinoline derivatives is tested. These 131I labeled quinolines are known to concentrate in malignant melamona. In an interdisciplinary approach these compounds are synthesized and evaluated. Also the tumor seeking agent 67-gallium citrate is presently used for this investigation. Furthermore, efforts have been made to repair a cell line of the Greene melanoma and to isolate melanin in order to be able to perform in vitro studies without having to sacrifice animals.

86. AN INTEGRATED SYSTEM FOR THE QUANTITATIVE ANALYSIS OF AXIAL TOMOGRAPHS OF PATIENTS WITH CEREBRAL EDEMA

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OBJECTIVE: Development of computer-based system for quantitative analysis and display of EMI Scanner tomographs,

incorporating computation of density statistics and volume of specified regions of interest. Angular reconstructions of the original data sets to facilitate tomographic views of any plane, and the application of this system to monitor the effects of various treatments (steroid therapy with/without tumor excision with/without irradiation) on cerebral edema.

APPROACH: 1) In cooperation with Hull University (Depts. of Computer Studies and Electronic Engineering), development of image display system similar to that on the EMI Scanner. 2) Writing of software package comprising a set of programs enabling the user to retrieve/modify/store scan data, display and annotate individual scans and further analyze data - picture subtraction, area/volume analysis, density profiles, angular reconstuctions. 3) Collation of clinical data (from Hull, R.I., Atkinson Morley's Hospital, Royal Marsden Hospital) on approximately 100 patients suffering from malignant brain tumors and 60 patients with cerebral edema.

PROGRESS: 1) Image display system completed - slave monitor and camera to be added. 2) All 2-dimensional scan analysis implemented -analysis of volumes under development. 3) Approximately 20 percent of clinical data acquired.

87. TOPOGRAPHY OF BRAIN TUMORS - COMPUTERIZED TRANSFER OF COORDINATES FROM RADIOLOGIC PROCEDURES (ABBREV)

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This project aims at the development of a patient head fixation device combined with modalities for exact delineation of the location and spread of brain tumors in order to improve their radiological and surgical treatment. This system will allow exact and automatic transfer of spatial information from a diagnostic situation (computer tomography, plain skull radiography, conventional tomography, angiography, pneumoencephalography, brain scintigraphy, positron emission tomography, etc.) to a therapeutic situation (biopsy, puncture of cystic lesions, radiosurgery, radiotherapy, etc.).

88. COMPUTED TOMOGRAPHY (TRANSMISSION)

Di Chiro, G., Brooks, R. A., Rieth, K. G., Sank, V. J., Kornblith, P. L., Smith, B. H., Quindlen, E., Herdt, J. R., Vermess, M., Johnston, G. S., Jones, A. E., Kessler, R. M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Neurological & Communicative Dis. & Stroke, Neuroradiology & Computed Tomography Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Computed Tomography (CT), in its transmission and emission modalities, represents the main research area of the Neuroradiology and Computed Tomography Section.

Ongoing clinical research projects in transmission CT deal with the following pathological processes: degenerative, demyelinating and atrophic processes of the brain; hydrocephalus, brain edema; postradiation cerebral necrosis; surgically correctable lesions in young patients affected by chronic epilepsy; diseases of the spine and the spinal cord; attempts at tissue characterization of normal and abnormal (e.g., tumoral) cerebral tissue.

PHYSICS PROJECTS: Improved dual-energy CT scanning using both a split-detector and a dual kVp method; analysis of aliasing effects and developments of methods for their elimination; phantom studies for the evaluation of artifacts and calibration of CT machines. Feasibility tests to build a new type of CT device which will use protons are under way.

BIBLIOGRAPHIC REFERENCES: Arimitsu, T., Jabbari, B., Buckler, R.E., and Di Chiro, G.: Computed tomography in a verified case of tuberculous meningitis. Neurology 29-384-386, 1979. Di Chiro, G., Arimitsu, T., Brooks, R.A., Morgenthaler, D.G., Johnston, G.S., Jones, A.E., and Keller, M.R.: Computed tomography profiles of periventricular hypodensity in hydrocephalus and leukoencephalopathy. Radiology 130-661-666, 1979.

89. CEREBRAL BLOOD FLOW WITH STABLE XEONON CMPTR TOMOGRAPHY

Hilal, S. K., Kelcz, F., Joseph, P. M., Columbia University, College of Physicians & Surgeons, Dept. of Radiology, *630 W. 168th St., New York, New York*, 10032, United States of America

The measurement of regional cerebral blood flow requires simultaneous measurement of the xenon clearance rate and the brain-blood partition coefficient. However, in the usual nuclear medicine study only the xenon-133 clearance is measured and the blood flow is calculated using published values of the brain-blood partition coefficient for normal tissue. This may lead to large errors in the case of pathological tissue because the partition coefficient varies greatly in brain tumors. In addition, scatter, poor localization and non-uniform sensitivity with distance from the detector hamper the usual studies.

The computerized tomographic (CT) scanner is a new imaging device that yields a cross-sectional view of the body with very high sensitivity to slight changes of radiodensity in the various body tissues. We have already shown that the CT scanner may be used to quantitate the amount of stable xenon in the brain. We plan to use this ability to measure the local partition coefficient in vivo. With the animal (and later the patient) inhaling a xenon mixture, one waits for saturation of the brain by xenon. A scan at this time will allow a map of the partition coefficient in the various brain tissues to be produced. Returning the animal (or patient) to oxygen inhalation and taking scans during the subsequent washout should allow us to use the local partition coefficient and clearance to produce, for the first time, a perfusion image of the brain. We intend to use this method in adult patients with brain tumors and adult patients with advanced occlusive vascular disease. It is felt that these two groups can justifiably be exposed to the small radiation hazard involved in this method. We expect to obtain accurate measurements for 0.5 cc of brain.

BIBLIOGRAPHIC REFERENCES: Kelcz, F., Hilal, S.K., Hartwell, P., Joseph, P.M.: CT measurement of the xenon brain-blood partition coefficient and implications for rCBF: A preliminary report. Radiology 127: 385-392, 1978 (May).

90. CLINICAL EVALUATION OF COMPUTERIZED TRANSAXIAL TOMOGRAPHY

Potts, D. G., Cornell University, Medical Center, Cornell Medical College, Dept. of Radiology, *1300 York Ave., New York, New York*, 10021, United States of America

OBJECTIVES: Clinical evaluation of the use of computerized transaxial tomography to determine the value of the EMI apparatus in the diagnosis of brain tumors, especially as compared to angiography and pneumoencephalography; specifically, to attempt to define the capability of the scanner to predict earlier lesions, cystologic type, and the ability to localize lesions. This study will also determine whether there is a clinical presentation for which either the EMI or nuclide brain scan is the preferable initial diagnostic imaging study, and for which angiography and/or pneumoencephalography is also to be desired. Also to determine absorption data in normal patients.

91. RADIOGRAPHIC IMAGING FOR CANCER DIAGNOSIS

Doi, K., Kirchner, P. T., Metz, C. E., Duda, E. E., Anderson, T. M., MacMahon, H., Patronas, N. J., Kodera, Y., Vyborny, C. J., University of Chicago, Pritzker School of Medicine, Dept. of Radiology, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

The aim of our research is to improve the diagnostic quality of radiographic images for cancer diagnosis and to minimize the patient exposure incurred during the radiographic examination. Our plan includes (1) application of magnification radiography with an ultra high speed rare-earth recording system to chest radiography for the characterization of pulmonary masses (and to distinguish benign from malignant); (2) in bone radiography, development of a high speed and high resolution technique for skeletal radiography to identify bone metastases and to assess disease progression; and (3) evaluation of new stereoscopic techniques for accurate diagnosis of brain tumors. Clinical results will be analyzed by a group of radiologists in blind studies or side-by-side comparisons of radiographs, in order to establish statistically the usefulness of the new techniques applied to various examinations.

The technical approach to applied and clinical studies of radiographic imaging described above is based on the application of methods of optical communication theory to the analysis of factors affecting detail visibility of radiographic images. This involves (1) x-ray energy spectral analysis to study the relationship between the beam quality, patient exposure and image quality; (2) sensitometric evaluation of recording systems; (3)

evaluation of modulation transfer functions to provide transfer characteristics of recording systems and of geometric unsharpness; (4) investigation of radiographic mottle to determine the effect of noise on detail visibilities of low contrast radiologic objects; (5) computer simulation studies to optimize the physical parameters discussed above, and (6) ROC analysis of observer performance in detecting simple test object images and in evaluating diagnostic accuracies of bone radiography.

BIBLIOGRAPHIC REFERENCES: Chan, H.P., and Doi, K.: Investigation of scattered radiation in diagnostic radiology by Monte Carlo method. Medical Physics 7: 422, 1980 (abstract). Doi, K., and Chan, H.P.: Evaluation of absorbed dose in mammography: Monte Carlo simulation studies. Radiology 135: 199-208, 1980.

92. CLINICAL DEVELOPMENT OF RADIOPHARMACEUTICALS

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The task of this proposal is to apply new radiopharmaceuticals and new scanning techniques to the diagnosis of human diseases and make in vivo studies to evaluate physiological processes that are altered in occupational diseases of the respiratory and the gastrointestinal tract. The radiopharmaceuticals used in this task are primarily positron emitters suitable for detection by coincidence counting with an emission computerized tomography (ECT) scanner designed to measure activity distribution in cross-sectional slices through selected levels of the body. Emphasis is placed on studying amino acid utilization and metabolism in malignant tumors of the pancreas and the brain with 11C-carboxyl-labeled DL-tryptophan and DL-valine, and the unnatural amino acids 11C-aminocyclopentanecarboxylic acid and 11C-aminocyclobutanecarboxylic acid. ECT with 68Ga-labeled radiotracers such as albumin microspheres, EDTA, red blood cells, and platelets is used to study lung function, lung diseases, and neoplasms. Applications of ECT in our clinical investigations are dependent upon and supported by assistance and active research in computer programming and development of new computer applications in ECT and conventional scintigraphic imaging.

BIBLIOGRAPHIC REFERENCES: Huebner, K.F., et al. Differentiation of Pancreatic and Other Retroperitoneal Tumors by Positron Emission Computerized Tomography (ECT),J. Nucl. Med. 20: 631 (1979). Huebner, K.F., et al. Carbon-11-Labeled Amino Acids for the Rectilinear and Positron Tomography Imaging of the Human Pancreas, J. Nucl. Med. 20: 507-513 (1979).

93. MELANOMA DETECTION WITH RADIOPHARMACEUTICALS

Atkins, H. L., Packer, S., Wolf, A. P., Lambrecht, R. D., Christman, D., Fairchild, R., Brookhaven National Lab., *Upton, New York*, 11973, United States of America

The clinical usefulness of the melanin-seeking radiopharmaceutical iodine-123 DMQ (4,3 dimethylamino-propylamine-7-iodoquinoline) is being determined. Specifically, we are looking at patients with malignant ocular (choroidal) and skin melanomas.

New detection devices include: 1) a dual pinhole eye collimator for use with a gamma camera, 2) a two eye-probe counting system (this allows simultaneous counting of 2 eyes), and 3) an ultrasound guided gamma probe (on loan from the University of Michigan). Large melanomas (1000 mm3) have been imaged but we do not know the limitations of the systems mentioned above, since too few patients have been studied. Patients are also being similarly studied with gallium-67 citrate.

Laboratory investigations into the role of melanoma-localizing agents pre- and post-irradiation continue. We are using the Greene malanoma in the rabbit and gallium-67 citrate before and after irradiation with iodine-125.

Our immediate goal has been to find a non-invasive method of determining the presence of an intraocular (choroidal) melanoma and then to evaluate the effects of radiotherapy and/or hyperthermia with melanoma-seeking radiotherapy and/or hyperthermia with melanoma-seeking radiopharmaceuticals.

94. RADIOIODINATED CHLOROQUINE ANALOG FOR DIAGNOSIS OF OCULAR MELANOMA

Gaasterland, D. E., Ballintine, E. J., Kupfer, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Eye Inst., Clinical Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this clinical investigation has been to assess the value of systemically administered 125I-labelled chloroquine analog for the detection of ocular melanoma. Patient enrollment terminated June 30, 1975, after the 36th patient was accepted. Follow-up examinations of some patients continue. This is expected to yield information on the clinical course of diagnosed and treated melanoma patients who refused treatment, and of patients with lesions which may or may not be ocular melanoma. The course will be compared to the results of the radioactive tracer testing.

95. CERENKOV DETECTOR SUBCOMPONENT

Price, J., U.S. Veterans Admin., Dept. of Medicine & Surgery, Outpatient Clinic, *Lubbock, Texas, United States of America*

A Cerenkov detector is under development as a non-invasive technique for the detection of intraocular melanomas. It may also be used as a system for studying hard-beta emitter kinetics in the eye. A quantum efficient photo-multiplier detection system is under development for this purpose. Initial calibration and construction have been completed. A study is under way to select an optimal coupling system. The first animal studies are being initiated.

96. EVALUATION OF DIPHENYLHYDANTOIN-11C AS A BRAIN SCANNING AGENT

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This is part of a broader project. A summary of this subproject is not available.

D. OTHER DIAGNOSTIC METHODS

97. NEUROCOMPUTER PROJECT

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OBJECTIVE: To diagnose site and type of intracranial tumor by means of the memory core of a computer.

APPROACH: The clinical signs and symptoms were coded into numbers from look-up lists of patients with a proven tumor. All radiological data, surgical details, radiotherapy treatment programs, the EEG and static brain scan, and the histology and progress of the patient were similarly treated.

PROGRESS: As a pilot scheme, new cases were matched against the memory bank for clinical data and radiological data only. A very high rate of successful prediction was achieved. The mathematical basis has to be further tested when more cases are added to the bank. Preliminary subsidiary paper is in press dealing with information relating to a separate item that arose as the main work was in progress - namely, diagnosis of posterior fossa tumors using the plain skill radiograph alone. Unfortunately, normal data were not available so the work to obtain this was undertaken first using a grant from S.C.G. Hospital.

FUTURE: 1. Computerized axial tomography (EMI scanner) will be used to add further to the interrelating radiological and clinical data. 2. The natural history of various forms of hydrocephalus is to be studied with special emphasis on that mechanism operating in obstructive hydrocephalus of tumor.

98. STEREOTACTIC BIOPSIES IN GLIOMAS

Boethius, J., Collins, V. P., Edner, G., Bergstrom, G., Karolinska Institutet, Dept. of Neurosurgery, *Solnavagen 1, S10401 Stockholm 60, Sweden*

Multiple stereotactically placed biopsies are taken from gliomas. The stereotactic localization of the biopsies is determined directly with aid of CT-scanning. At present the correlation between pathological anatomy, cytology, thymidine incorporation and in vitro properties is determined separately for each one of the multiple biopsies. The project is incorporated in the clinical routine of the department.

99. ULTRASONIC EVALUATION OF OCULAR TISSUES

Lizzi, F. L., Feleppa, E. J., Greenebaum, M., Herbst, J., Rosenberg, S., Jaremko, N., Riverside Research Inst., Biomedical Engineering Laboratory, *80 W. End Ave., New York, New York,* 10023, United States of America

The overall objective of the present program is to provide the comprehensive improvements in opthalmic diagnosis and treatment monitoring which are needed to permit the optimal use of current and future therapeutic methods. The program is exploring advanced ultrasonic spectrum analysis techniques and testing them for reliability in a working clinical system. The objective is to attain specificity of tissue diagnosis utilizing noninvasive ultrasonic methods. Characteristic histologic features are being assessed by 'signatures' involving several sets of ultrasonic parameters. Precision of tissue characterization will enable detection and identification of a broad class of disorders including, most importantly, intraocular and orbital tumors. Tissue signatures are being determined from an advanced ultrasonic diagnostic system using minicomputer technology and digital processing techniques including deconvolution and cepstrum analysis.

A computer library of these spectral parameters is being developed to serve as the focal point of efforts to identify sets of characteristics associated with specific diseases. Concurrently, experience gained with on-line clinical processing is being used to formulate techniques and diagnostic protocols which will allow the most efficacious use of these signatures in arriving at diagnostic decisions.

The research is a collaborative effort of clinical and engineering staffs. Engineering efforts have been and will continue to be performed at Riverside Research Institute. The clinical studies studies will be performed at the Dept. of Ophthalmology, Cornell University Medical College.

BIBLIOGRAPHIC REFERENCES: Lizzi, F.L., Coleman, D.J., Franzen, L., and Feleppa, E.J., Use of a Spectrum Analysis System for Characterization of Malignant Melanoma, Ultrasound in Medicine, Vol. 4, ed. D. White and E.A. Lyons, Plenum Press, New York, pp. 559-562, 1978. Lizzi, F.L., Coleman, D.J., Driller, J., Franzen, L.A., Jakobiec, F.A., Experimental Ultrasonically Induced Lesions in the Retina, Choroid and Sclera. Invest. Ophthal. 17:350-360, 1978.

100. ULTRASONIC EVALUATION OF OCULAR TISSUES

Coleman, D. J., Franzen, L. A., Smith, M. E., Simoni, G. J., Jakobiec, F. A., Cornell University, Medical Center, Cornell Medical College, Dept. of Ophthalmology, *1300 York Ave., New York, New York,* 10021, United States of America

The overall objective of the present program is to provide the comprehensive improvements in ophthalmic diagnosis and treatment monitoring which are needed to permit the optimal use of current and future therapeutic methods. The program is exploring advanced ultrasonic spectrum analysis techniques and testing them for reliability in a working clinical system. The objective is to attain specificity of tissue diagnosis utilizing noninvasive ultrasonic methods. Characteristic histologic features are being assessed by 'signatures' involving several sets of ultrasonic parameters. Precision of tissue characterization will enable detection and identification of a broad class of disorders including, most importantly, intraocular and orbital tumors. Tissue signatures are being determined from an advanced ultrasonic diagnostic system using minicomputer technology and digital processing techniques including deconvolution and cepstrum analysis.

A computer library of these spectral parameters is being developed to serve as the focal point of efforts to identify sets of characteristics associated with specific diseases. Concurrently, experience gained with on-line clinical processing is being used to formulate techniques and diagnostic protocols which allow the most efficacious use of these signatures in arriving at diagnostic decisions.

The research is a collaborative effort of clinical and engineering staffs. Engineering efforts have been and will continue to be performed at Riverside Research Institute. The clinical studies will be performed at the Dept. of Ophthalmology, Cornell University Medical College.

101. IMPROVED DIAGNOSIS AND THERAPY IN OPHTHALMOLOGY

Zimmerman, L. E., McLean, I. W., Font, R. L., Fine, B. S., Universities Associated for Research & Educ. in Pathol. Inc., Dept. of Ophthalomic Pathology, *9650 Rockville Pike, Bethesda, Maryland,* 20014, United States of America

We propose to continue and/or complete the projects still in progress and to undertake new projects. Studies of prognostic factors in melanotic tumors of the iris and retinoblastoma have been proposed.

In our continuation of cytologic evaluation of uveal nevi and melanomas, increased use of electron microscopy will be made in an effort to further refine the Callender classification and to assist in defining better the criteria for differentiating spindle cell nevi from spindle cell melanomas. Electron microscopy will also be employed to study the integrity of the vascular walls of dilated blood vessels that are so characteristically present, especially in the larger melanomas of epithelioid or mixed cell type that frequently prove lethal after, but almost never before, enucleation. Comparisons will be made between the electron microscopic appearance of the vascular walls in eyes subjected to standard procedures of enucleation and those enucleated by the 'no touch' technique.

BIBLIOGRAPHIC REFERENCES: Gamel, J. W., McLean, I.W., Foster, W.D., and Zimmerman, L.E.: Uveal melanoma. Correlation of cytologic features with prognosis. Cancer 41: 1897-1901, 1978. McLean, I.W., Zimmerman, L.E., and Evans, R.M.: Reappraisal of Callender's spindle A type of melanoma of choroid and ciliary body. Am. J. Ophthal. 86:557-564, 1978.

102. LONG-TERM MEASUREMENT OF INTRACRANIAL PRESSURE BY MEANS OF AN IMPLANTED HITTMAN-MEYER INTRACRANIAL PRESSURE SENSOR

Kaplan, R. J., Pollay, M., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Surgical Service, *921 N.E. 13th St., Oklahoma City, Oklahoma,* 73104, United States of America

PROJECT OBJECTIVES: To test the reliability and accuracy of the Hittman-Meyer Intracranial Pressure Transducer in the measurement of intracranial pressure on a chronic basis in human patients.

PLAN: The pressure transducer will be implanted in patients harboring neurological lesions, which produce elevated intracranial pressure, who are expected to survive in excess of six months. Patients with chronic elevated intracranial pressure caused by such conditions as pseudotumor cerebri, complex hydrocephalus or unresectable benign intracranial tumors, will be considered candidates.

METHOD: The intracranial transducer is implanted through a burr hole into the subdural space at the time of the patient's primary operation or as a separate operation.

FINDINGS: The device has now been tested in two patients over a period of two years. It has given consistent reproducible results which have been checked against pressures measured intermittently by lumbar puncture and has revealed no evidence of chronic drift of the measurements although in one case there was a 7 millimeter of water pressure difference between the measurements made via the transducer and those made by lumbar puncture. This was a consistent difference and is not felt to significantly interfere with the usefulness of the device.

103. AUTOMATED NONINVASIVE DIAGNOSIS OF BRAIN DYSFUNCTION

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Methodology for the objective non-invasive diagnosis of functional and anatomical brain lesions will be developed. Brain electrical potentials from multiple scalp electrodes will be recorded from patients with supratentorial brain tumors and from normals. Spectral analysis will be performed on resting electroencephalographic activity and sensory evoked potentials will be formed. Topographic maps of the spectral and evoked potential data will be created. An interactive development and evaluation of features descriptive of the topographic, spectral,

and dynamic course of brain electrical potentials will be followed by automated information extraction and computer processing of features by self-learning computer algorithms. Sensitive measures of symmetry of feature distributions will be employed. Objective characterizations of the locations and size of brain tumors (CAT scan) will be derived from the topographic features. Objective characterizations of the location and magnitude of the associated functional deficits (neuropsychological testing) will also be derived. A data base of topographic images will be collected and a subcategorization of different diagnostic categories, based on objective criteria, will be established. This automated diagnostic methodology will be compared to more conventional diagnostic techniques.

104. CLINICAL RECORDING OF SACCADES

Bahill, A. T., Troost, B. T., Carnegie-Mellon University, Carnegie Inst. of Technology, Dept. of Electrical Engineering, *Frew Ave. & Margaret Morrison, Pittsburgh, Pennsylvania,* 15213, United States of America

The shapes of saccadic eye movement tracking patterns have been used to aid the diagnosing of certain disease states, such as myasthenia gravis, multiple sclerosis and cerebellar tumors. However, some of these studies may have been confounded by fatigue, lack of normalitive data, and instrumentation difficulties. We propose to gather normalitive data about the variations in the hyperfine structure of saccadic eye movements and to use this data gathered with the same instrumentation to study patients in a new laboratory.

A specific hypothesis to be tested is that the presence of glissadic undershoot of the adducting eye and concomitant glissadic overshoot of the abducting eye along with abduction nystagmus is not a sufficient test for internuclear opthalmoplegia nor is it a good early diagnostic for multiple sclerosis. We wish to identify the control signal variations that produce these glissades.

105. ANALYSIS OF ELECTROPHYSIOLOGICAL DATA IN CENTRAL NERVOUS SYSTEM DISORDERS

Flanigin, H. F., Flanigan, S., Boop, W. C., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *300 E. Roosevelt Rd., Little Rock, Arkansas,* 72206, United States of America

The activity of this project has been seriously hampered by lack of technical personnel support. Some activity, however, has been possible using borrowed technicians from the EEG laboratory and voluntary personnel from the Univ. of Ark. for Medical Sciences.

During this past year, in collaboration with the Audiology Service, an Evoked Potential Laboratory was established and equipped with basic instrumentation. In the 3 months of operation with the help of volunteers, 46 evoked potential studies were done and many more have been requested. These studies have been of aid to requesting services in diagnosis and management, and further research development is anticipated.

Intraoperative EEG monitoring has continued to be used in carotid artery surgery for assessment of cerebral circulatory function. Such studies on 18 patients during the past year indicate its continued value in determination of intraoperative ischemic changes.

Electrocorticography has been carried out in selected patients with cerebral irritability due to neoplastic or cicatricial lesions. Cortical stimulation and functional mapping have been performed for identification and preservation of function in selected patients.

106. OCULAR TUMOR DIAGNOSIS

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The goal of this project for the coming year is to carry out a clinical study of orbital disease using a new ophthalmic ultrasonoscope with digital recording capabilities, and to compare the results obtained with this ultrasonoscope with those achieved by computerized axial tomography of the orbit.

107. CLINICAL AND EEG FEATURES IN CASES OF MIDLINE (PARASAGITTAL) EPILEPTIFORM ABNORMALITIES AND PATHOLOGIC LESIONS

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A correlative study of the clinical, electrographic and seizure patterns of 20 pathologically proven midline (parasagittal) lesions has been carried out. A similar group of 30 cases with electrographic interictal and ictal epileptiform discharges originating from the vertex region (CZ electrode) has been selected for comparison of their seizure pattern and behavior to differentiate this as a group from the tumor cases.

108. PRIMARY INTRAORBITAL MENINGIOMA - PROBLEMS IN DIAGNOSIS AND MANAGEMENT

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The purpose of this study is to determine whether there are significant differences in the behavior and prognosis of orbital meningiomas in children, in young adults, and in middle-aged or older adults.

Well-documented cases on file in the Registry of Ophthalmic Pathology were selected for follow-up study. Every effort was made to obtain current follow-up data. When there was a history of local recurrence or intracranial involvement requiring biopsy or surgical intervention, efforts were made to obtain representative sections for histopathologic study.

It appears that meningiomas of childhood carry the worst prognosis while those of older individuals carry a guarded prognosis.

III. PRECLINICAL THERAPY OF NERVOUS SYSTEM TUMORS

A. CHEMOTHERAPY

109. PHARMACOLOGICAL CONTROL OF HYPOPHYSEAL HORMONES POSSIBLY INVOLVED IN CARCINOGENESIS AND NEUROENDOCRINE EFFECTS OF ANTICANCER DRUGS

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OBJECTIVE: Antineoplastic drugs can produce changes in neuroendocrine function in addition to their antitumor effects. These changes are of interest because alterations in endocrine states may reinforce or impair the cytotoxic effects of the antineoplastic compounds. The aim of the project is to investigate the neuropharmacological control of anterior pituitary hormones implicated in the tumorigenesis and the possible neuroendocrine interferences of various antineoplastic agents, whether they cross the blood brain barrier or not.

APPROACH: We have employed stereotaxic and microsurgery techniques in order to infuse in the cerebral ventricles or to implant in different brain areas antineoplastic agents or pharmacological tools in order to investigate central neurotransmitter functions in the control of anterior pituitary hormones. Using these procedures we have evaluated or will evaluate the circulating levels (by RIA) of anterior pituitary hormones like TSH, prolactin, ACTH, corticosterone secretion and some behavioral parameters. Antineoplastic agents crossing the blood-brain barrier were or will be given also by the clinically most commonly employed administration route.

PROGRESS: The experiments investigating the role played by central serotonin (5HT) neurons in the secretion of prolactin, TRH-TSH and ACTH have shown that 5HT can stimulate prolactin and ACTH release; on the other hand, this neurotransmitter exerts an inhibitory function on TRH-TSH secretion. Concerning the studies on neuroendocrine interference of antineoplastic drugs we found that the fluorinated pyrimidine 5FU stimulates corticosterone secretion from the adrenal cortex. In order to correlate this effect with the biochemical action of 5FU, thymine was administered to rescue the neuroendocrine

effect of 5FU. The results show that thymine was ineffective in this regard, but it should be considered that only one dose of thymine was utilized and therefore more detailed studies are necessary to clarify this point. At present experiments on the effects of methotrexate, procarbazine and hydroxyurea on brain amines and anterior pituitary hormones are in progress.

110. SUPERDOSIS CANCER CHEMOTHERAPY OF HUMAN TUMORS GROWN IN NUDE MICE WITH DIMETHYL MYLERAN AND AUTOLOGOUS BONE MARROW INFUSION

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OBJECTIVES: To test the sensitivity of human tumors grown in nude mice to superdosis chemotherapy with dimethyl Myleran followed by bone marrow transplantation.

APPROACH: Human tumors, including melanomas, hypernephromas, bronchial carcinomas and colon carcinomas, are serially maintained in nude mice. Panels of nude mice carrying these tumors are subjected to lethal doses of dimethyl Myleran (DMM) followed by isogeneic bone marrow. Tumor response and survival are followed. In particular, we are now interested in determining whether individual tumors of the same type display a heterogeneic or a similar sensitivity to this chemotherapeutic protocol. If the latter is the case, the intended therapeutic scheme could be applied clinically without testing the sensitivity of every single tumor to DMM beforehand. Moreover, it is planned to study whether the lethality of tumor-eradicating doses of DMM can be overcome also by autologous bone marrow transplantation.

PROGRESS: Several but not all of the tested tumors can be completely eradicated with the above scheme. The effect of supplementary submarginal T-cell doses was also studied. A preliminary report has been published.

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111. POSSIBLE ROLE FOR LIPOPHILIC CNS DRUGS IN CANCER CHEMOTHERAPY

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We find that eight benzodiazepine tranquilizers hold cultured 3T3 cells out of S-phase in high serum medium (See Report 194). Evidence points to a plasma membrane site for this action. Tumor cells, less dependent on exogenous polypeptides (i.e., with an endogenous means of maintaining high transition probabilities) might be less easily held. Any lipophilic drug, in addition to effects on nerve, muscle, etc., might hold bone marrow and gut cells out of S-phase in patients treated with S-phase specific anticancer drugs (MTX, Ara C and hydroxyurea in particular) without a reduction in their potency.

We are testing this proposition in mice and exploring the possibility of its gradual assessment in patients.

The possible relevance of our findings to the theoretical effects of prolonged exposure to lipophilic drugs on occurrence of congenital defects, tumor progression and development of immunological deficiency is obvious. Such associations have been claimed in the literature, but previously no mechanism has to our knowledge been proposed.

112. INFLUENCE OF ANTICOAGULATION ON METASTASIS FORMATION

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OBJECTIVE: Analysis of the process of adherence of tumor cells to the vascular endothelium: role of fibrin, evaluation of the effect of anticoagulant drugs on the distribution and survival of circulating tumor cells, analysis of mechanism of action; evaluation of possible clinical application of anticoagulative therapy in cancer treatment.

APPROACH: Four spontaneously metastasizing tumor systems in rats and mice (Lewis lung-B16-WAG rat Schwannoma-SD astrocytoma) are systematicaly screened for metastasis distribution and formation during various anticoagulant regimens (heparin, coumarins, fibrinolytic agents, etc.). The effect of anticoagulation is being studied after tumor cells are injected i.v. and transplanted s.c. Furthermore, the possibility of direct drug-tumor cell interactions will be studied.

PROGRESS: Administration of heparin or fibrinolytic agents drastically reduces the number of experimental lung tumor colonies after i.v. inoculation of tumor cells. Heparin also causes an increase in extrapulmonary, extrathoracic colonies. In contrast, spontaneous metastasis formation is not influenced. Coumarin derivatives cause a reduction in both lung tumor colonies after i.v. inoculation of cells and spontaneous metastases from s.c. transplanted tumors. Literature and experimental data indicate that the mechanism of action of coumarins is independent of coagulation factors. Restoration of the induced coagulation impairment by adding factors II, VII, IX and X inhibits the coumarin-induced reduction of the number of lung tumor colonies. Selective macrophage inhibition appeared to abolish the coumarin-induced reduction of spontaneous metastasis. Further emphasis will be placed on the analysis of the role of vitamin K and macrophages.

113. THE DEVELOPMENT OF DRUG-RESISTANT CELL LINES IN CULTURE

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Drug resistance remains a major problem in human cancer chemotherapy. With many of the drugs that are considered effective in the treatment of human tumors, remissions or objective regressions of 'solid' tumors, though dramatic, are often of short duration and resistance to subsequent therapy is observed. A knowledge of which drugs can overcome such resistance is therefore essential. We are investigating whether certain mammalian cell lines exhibit cross-resistance to different types of drugs. In addition, we are screening for agents specifically effective against drug-resistant cell lines developed in this laboratory.

Initially, we have used the L5178Y mouse lymphoma line and by cloning techniques have successfully developed and maintained lines that are resistant to (1) methotrexate, (2) 5-fluorouracil, (3) methotrexate and 5-fluorouracil, (4) vincristine, and (5) vindesine. Our results with the methotrexate-resistant lines have been reported previously (ICRF Scientific Report 1977 - report 115 and ICRF Scientific Report 1978 - report 14). Quelamycin, a newly developed anthracycline antibiotic, shows promise against 5-fluorouracil-resistant cells, whilst preliminary data indicate some lack of cross-resistance between vincristine and vindesine.

We are currently attempting the development of lines resistant to other drugs commonly used in clinical practice, such as adriamycin and cis-platinum. In addition, we are trying to establish resistance in tumor lines of human origin derived from neuroblastomas, colonic carcinomas and squamous cell head and neck cancers.

114. NEUROPATHOLOGICAL INVESTIGATION OF THE EFFECTS OF METHOTREXATE ON BRAIN TUMORS

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OBJECTIVES: Investigation of the influence of methotrexate (MTX) on the growth of brain tumors in tissue culture. Morphological and enzyme histochemical changes in tissue sections are also studied. Special attention is paid to the function of monocytes and macrophages in brain tumors.

115. RADIOLABELING TECHNIQUE FOR HUMAN TUMOR STEM CELL ASSAY

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Advanced bronchogenic carcinoma, colorectal carcinoma, breast carcinoma, and neuroblastoma account for a significant number of deaths per year from cancer in the United States. A recent advance which would allow the correct choice of chemotherapy for an individual patient with one of these malignancies has been the development of the human tumor stem cell assay by Hamburger and Salmon. However, at the present time the assay presents several problems which must be solved before the system can be used for widespread patient care applications. One major problem is that with the present methodology it takes 21 days for colonies to grow before they can be counted

and sensitivity to chemotherapy determined. Three weeks is too long to wait before starting an anticancer agent in most clinical situations. A second major problem is that the assay system consumes such a large amount of technical time for counting colonies (10 hours per tumor specimen) that the assay system is not practical even for institutions with reasonable technical support. The first phase of this research proposal addresses these two problems by developing a technique that will provide an early answer (6 days or less) as to the number of colonies which survive in vitro drug treatment. This would give the clinician an early indication of drug sensitivity of the tumor. This radiolabeling technique would also obviate the need for colony counting which would mean a great saving of time. This radiolabeling technique could also provide a powerful tool for studying the kinetics, metabolism, drug transport and resistance of human tumor stem cells through the use of a variety of labeled proteins and drugs. The second phase of this proposal stresses the utilization of the radiolabeling technique (developed in phase I) to study the growth kinetics of the human tumor stem cell. It is hoped that these studies of what is potentially the proliferating compartment of the tumor will lead to a better understanding of the tumor kinetics and design of clinical trials to exploit this understanding for treatment purposes.

116. INHIBITION OF PTERIDINE ENZYMES IN CANCER CHEMOTHERAPY

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Although a potent inhibitor of dihydrofolate reductase, methotrexate (MTX) is effective only against certain forms of cancer, and even these may acquire resistance. Furthermore, since MTX cannot cross the blood-brain barrier, treatment of meningeal leukemia and brain tumors necessitates intrathecal or intraventricular injection. However, therapeutic levels of MTX in the brain often result in symptoms of neurotoxicity, the cause of which is unknown. These limitations have stimulated the search for alternative anti-folates. The objective of this proposal is twofold. First, to determine the molecular basis of specificity of pteridines and related compounds so that anti-folates may be designed which are specific for dihydrofolate reductase and which do not interfere with other pteridine utilizing enzymes. Series of substituted pteridines and pyrimidines will be studied as cofactors and inhibitors of the three aromatic amino acid hydroxylases. These enzymes have a specific requirement for a pteridine cofactor, tetrahydrobiopterin (BH4), and are involved in the biosynthesis of serotonin and the catecholamine neurotransmitters. The effects of the pteridines and pyrimidines will also be assessed on dihydropteridine reductase, the enzyme which in vivo regenerates BH4. Any pteridine-like drug aimed at dihydrofolate reductase, including pyrimidines as recently demonstrated (Bailey and Ayling, Biochem. Biophys. Res. Comm. 85, 1614-21, 1978), has the potential to restrict BH4 dependent neurotransmitter synthesis. Second, to determine whether the toxicity of MTX, or its metabolites, is caused by inhibition of BH4 biosynthesis, transport, or maintenance. The levels of BH4 and its precursors in rat tissues will be monitored in response to MTX. An understanding of the molecular basis of anti-folate toxicity will enable counteractive measures to be taken. The structure-activity relationships will provide the information necessary to make drugs which selectively inhibit only dihydrofolate reductase.

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117. OSMOTIC OPENING OF BLOOD BRAIN BARRIER TO CHEMOTHERAPY

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There is presently not much evidence that chemotherapy is of benefit in malignant CNS tumors. The principal reason for this may be the existence of the blood brain barrier in the case of metastatic tumors whereas with regard to primary CNS tumors, drug sensitivity may be more important than the drug delivery problem. The presence of this barrier prevents most presently available chemotherapeutic agents from penetrating the CNS parenchyma and cerebrospinal fluid. This proposal is designed to try to overcome the problem of drug delivery with both animal and clinical studies. Hypertonic solutions have been shown by a number of investigators to disrupt transiently the blood brain barrier and has enabled agents such as microperoxidase, penicillin, Evan's blue labeled albumin, and various neurotransmitters which normally do not permeate the blood brain barrier to enter the central nervous system. Previous studies in this laboratory have shown that we can achieve a 10-to 50-fold increase in CNS methotrexate levels in the rat and dog after intra-arterial mannitol blood brain barrier disruption. No permanent neurologic defects have been noted. Close observation of the animals for up to two weeks following reversible barrier disruption has not revealed any neurologic complications. Neuropathologic examinations of rat brains after one day, seven days, and two weeks have not shown any evidence of histologic changes. Nonetheless, damage to the eye has been reported in some animals. Following barrier disruption C.T. scans of dogs which received intravenous conray 60 and intravenous Evan's blue before osmotic barrier disruption, show that there are marked areas of enhancement on the C.T. scan which correspond exactly to the areas of Evan's blue-albumin penetration of the blood brain barrier. There is a 20 C.T. density unit difference between the enhanced and unenhanced areas of the brain. The C.T. scan thereby is a sensitive non-invasive monitor of both the extent and the degree of blood brain barrier disruption. The present studies are planned to evaluate the effects of blood brain barrier disruption in man. We plan to determine the clinical and laboratory criteria documenting the presence of barrier disruption as well as the toxicity of barrier disruption. We then will evaluate the access of methotrexate or other drugs to malignant CNS tumors and the clinical responsiveness of such a chemotherapeutic maneuver.

118. METABOLIC PATTERNS AND CHEMOTHERAPY OF NEOPLASIA

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BACKGROUND: With present-day treatment, radiation and surgery are capable of curing approximately 50 percent of cancer patients; consequently, effective chemotherapy applicable to most or all cases of neoplastic diseases is urgently needed. Chemotherapy has already shown encouraging progress in the treatment of choriocarcinoma, Burkitt's lymphoma, certain other types of lymphomas and juvenile leukemias, Hodgkin's disease and certain endocrine tumors. However, in the great majority of solid tumors, adult onset leukemias, and especially brain tumors, chemotherapeutic efforts so far have failed to produce lasting results and there is a great deal to be achieved in the rational design of chemotherapy for cancer and allied diseases.

AIM OF THE RESEARCH PROGRAM: (1) The objective of this investigation is to elucidate strategic exploitable metabolic, enzymatic and isozymic differences between normal and neoplastic tissues of different growth rates and differentiation to provide a molecular basis for rationally planned selective enzyme pattern-directed experimental chemotherapy. (2) A thorough understanding of the main differences between normal and cancerous tissues at the molecular level should permit the evaluation of the regulatory mechanisms of gene expression which play crucial roles in the differentiation, replication and functioning of normal and neoplastic cells. It is expected that elucidation of the enzymatic basis of the key metabolic and regulatory alterations in cancerous tissues and an understanding of the factors which govern the pattern of gene expression might permit the design of new drugs and approaches to control neoplastic diseases by chemotherapy. (3) The ultimate goal of this program is to identify key metabolic, enzymatic and isozymic targets for chemotherapy and to develop chemical com-

pounds possessing a high degree of selective toxicity to cancer cells in order to achieve lasting chemotherapeutic results.

119. CSF DYNAMICS - CHEMOTHERAPEUTIC PERFUSATES

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This continuing program of research has shown that the brain-extracellular space-cerebrospinal fluid (CSF) diffusional pathway may be reversed for lipid-insoluble solutes so that chemotherapeutic agents may be administered to brain via CSF perfusion. Brain uptake of inulin plus methotrexate, at least, appears to be directly related to CSF perfusion pressure as well as the molecular size. We have shown that significant brain uptake of methotrexate at perfusion pressures below the CSF bulk absorption threshold does not occur. Simultaneous perfusions with inulin plus cytosine arabinoside and with inulin plus 8-azoguanine are underway to see whether these progressively smaller chemotherapeutic molecules will enter brain in more significant quantities. This study is also designed to observe the chronic effects of these antimetabolites on the neuropil of normal and hydrocephalic dogs. Our approach is to measure quantitatively and qualitatively the movements of high concentrations of these radioactive agents from cerebrospinal fluid to extracellular space by ventriculocisternal perfusion using rigid pressure controls. Brain uptake is measured by beta counting of serial biopsies and by calculation of the diffusional permeability coefficient of these agents as compared to inulin. We have shown that the rate of entry of inulin and of methotrexate into brain varies with perfusion pressure in acute animals and that even higher barin uptake occurs in the presence of subacute hydrocephalus. We have devised a technique in dogs that can be used to produce communicating, non-communicating or a combination of both types of hydrocephalus. A recent series of ventriculocisternal perfusions in dogs with subacute communicating hydrocephalus at 10 cm H2O pressure has resulted in significantly greater brain uptake of both methotrexate and inulin than in normal dogs perfused at pressures up to 15 cm of water. We propose to extend the project to investigate this phenomenon in dogs with chronic communicating hydrocephalus.

120. THE DISTRIBUTION OF DRUGS BETWEEN BLOOD, BRAIN, AND CEREBROSPINAL FLUID

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New techniques developed during this year include an astrocytoma tumor model, two ways of performing double label quantitative autoradiography, two approaches for analyzing blood-tissue transfer, and an awake animal ventriculocisternal perfusion procedure. Blood-tissue distribution studies of antitumor agents indicated that spirohydantoin mustard rapidly crosses the blood-brain barrier (BBB) and enters brain cells, that unchanged 14C-labelled AZQ does not penetrate the BBB as readily as expected, and that misonidazole easily exchanges across tumor and brain capillaries. Opening of the BBB and enhanced drug delivery to the brain would be achieved with intra-arterial infusions of hyperosmotic solutions but not with the inhalation of 20% CO2. In work with the metastatic Walker 256 brain tumor model, blood flow (BF) and glucose utilization (GU) were greatly reduced and blood-tissue transfer (BTT) was increased at foci within the large tumor masses; in tumor tissue around these foci, BF was low, GU was normal to higher than normal, and BTT was very high. For the small metastatic masses, GU was markedly increased, whereas BTT and BF were normal. For the oligodendroglioma model, BF, BTT, and GU were all normal or nearly normal for both large and small tumor masses.

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121. SUSTAINED-RELEASE CHEMOTHERAPY FOR OCULAR MALIGNANCY

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The purpose of this project is to develop an implantable chemotherapy system for sustained delivery of anticancer agents close to the site of a malignant intraocular tumor, in order to avoid the adverse effects of systemic administration. The initial work is focused on the effectiveness of nitrosourea compounds, particularly the lipophilic 1, 3-bis(2-chloroethyl)-1-nitrosourea (carmustine, BCNU), against a rabbit model of uveal Greene melanoma.

Topical application of BCNU on rabbit cornea was found to be more effective in raising the BCNU level in the aqueous humor than intravenous or subconjunctival injection. Release rate of BCNU from silicone balloons could be controlled by changing the surface area; in this way the dose can be adjusted. Combined topical application of BCNU on the cornea and intravenous injection of BCNU was found more effective in delaying growth of transplanted hamster Greene melanoma in the anterior chamber of rabbit eyes than either modality alone. The tumor was found to be capable of metastasis in the rabbit.

Goals for coming year: determination of diffusion rate of BCNU in the vitreous through a suprachoroidally implanted silicone device; determination of BCNU concentration in the aqueous after a silicone balloon has been fixed in the fornix and after a silicone contact lens has been placed on the cornea; evaluation of sustained release of BCNU, from a silicone device or a contact lens, for treating an animal model of rabbit with induced Greene melanoma in the eye.

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122. HUMAN GLIOMA HETEROGENEITY AND CHEMOTHERAPY

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Evidence exists that human brain tumors are composed of heterogeneous cell populations that account for their pleomorphism, biological change toward increased malignancy over time, and the occurrence of mixed gliomas and gliosarcomas. Such heterogeneity may also be responsible for the relatively poor results achieved in the chemotherapy of patients harboring malignant gliomas. To date, chemotherapy has usually been limited to single agents and this form of treatment may permit overgrowth of resistant cell lines. In our laboratory, different human malignant brain tumors growing in athymic nude mouse brains have displayed different chemosensitivities to standard agents, suggesting variability among gliomas and perhaps among different cell lines within each glioma. We propose to clone out these heterogeneous cell populations in tissue culture from gliomas obtained from patients undergoing resections of their tumors. The clonal lines will be characterized by morphology (light and electron microscopy), biochemical markers (S100 protein, glial fibrillary acidic protein, glycerol-3-phosphate dehydrogenase, cyclic nucleotide phosphohydrolase), karyology, growth kinetics, chemosensitivity, and cell surface markers. The parent lines and clones will be inoculated intracerebrally and subcutaneously into athymic nude mice to determine in vivo morphology, growth kinetics and chemosensitivity. The clone lines will be cocultivated to determine if phenotypic expression for drug sensitivity is altered and, if so, whether by intercellular exchange of diffusible substances and/or by cell-cell communication. Finally, utilizing the chemosensitivity profiles of the individual clones and information from cocultivation, new chemotherapy combinations will be designed and tested on the parent lines in vitro and in vivo. An attempt will be made to correlate chemosensitivities with other characteristics to find

early identifiable markers in order to suggest chemotherapy regimes for the patient as early as possible.

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123. SEARCH FOR A SPECIFIC THERAPY FOR NEUROBLASTOMA

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The broad aim of this research will be to develop a new chemotherapeutic agent for patients with neuroblastoma and related tumors of neural crest origin.

Media containing micromolar concentrations of partially oxidized catechols have been found to exhibit a highly selective toxic action on human and mouse neuroblastoma cells in culture. The same media added to other cell lines of embryological origin, other than neural crest, show consistently less toxicity.

In this application we propose to identify the product(s) formed from L-DOPA and other catechols, during the formation of melanin, which is toxic to cells of neural crest origin. This will be done by using paper and column chromatography of solutions of uniformly 14C-labeled L-DOPA, and other catechols as available, by testing the separated products for their toxic action on cells in culture, as indicated in the methods section of this application. Subsequently, following identification and accumulation of sufficient material, the action of the cytotoxic material(s) will be tested on the C1300 neuroblastoma in the mouse and on human neuroblastomas in nude mice, when these are available. The cytotoxicity of these same materials will be tested on other mouse and human tumors, not of neural crest origin, used as controls, to test specificity and sensitivity.

124. NEW APPROACHES TO ANTIFOLATE CHEMOTHERAPY

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Several classes of side-chain altered methotrexate (MTX) derivatives will be studied with the aim of obtaining novel antifolates with favorably modified biochemical, pharmacological, and therapeutic properties. Included in the program will be (a) lipophilic ester derivatives whose passive diffusion into cells offers a potential means of treatment of tumors that are MTX-resistant by virtue of impaired active transport, and whose nonpolar character increases their chance of being effective against tumors of the CNS; (b) amide and peptide 'prodrugs' that can undergo regionally selective biotransformation to MTX, either in specialized organs such as the liver or in the vicinity of tumors known to secrete amidases or peptidases; and (c) analogues of MTX in which the L-glutamate moiety has been replaced by other amino acids whose structure is designed to promote uptake into cells or bring about tighter binding to the target enzyme dihydrofolate reductase (DHFR). Test systems to be used in evaluating the new compounds synthesized during this program will include spectrophotometric enzyme inhibition assays using DHFR from normal liver and from tumor cells; measurement of (3H)-deoxyuridine and (3H)-thymidine incorporation into DNA and into the acid-soluble nucleotide pool; cytotoxicity assays using MTX-sensitive and MTX-resistant tumor cells in culture; antitumor assays against a variety of experimental tumor systems in animals; and distribution, biotransformation, and pharmacokinetic studies in various species such as mouse, dog, and monkey. Ultimately we hope to select a single compound whose biochemical and pharmacologic properties make it a logical candidate for clinical use. Once this has been done, appropriate pre-clinical studies will be performed and an IND application for a Phase I evaluation in man will be filed.

125. SYNTHESIS AND STUDY OF NEW CNS ANTITUMOR AGENTS

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The development of several highly lipophilic potential antineoplastic agents will be continued with the purpose of probing neoplasia of the CNS. The intent is to relate cytotoxicity and antitumor activity to the physicochemical properties of the agents in order to better define the requirements for therapeutic access to the CNS. Specifically, information is being sought regarding the characteristics of three related series of compounds (derivatives of azotricyclo-undecane), physicochemical information such as rates of hydrolysis and alkylation in situ, lipophilicity, and the nature of the transition state. Physicochemical correlations will be drawn within and between each series to establish a profile of each molecular system. Concomitantly, the biological and antitumor profile for each system will be determined utilizing an integrated approach consisting of in vivo kinetic profiles of drug entry into the cerebrospinal fluid and brains of rabbits. The detection and determination of the existence of passive diffusion and/or carrier-mediated transport, and the measurement of the effectiveness of subcellular localization in the CNS will also be accomplished. Antitumor screening will be carried out in in vitro KB-9 human nasocarcinoma cells, and samples will be submitted to NCI for in vivo screening. Using these data, correlations will be drawn which should afford new insights into the physicochemical requirements for CNS-active antitumor agents.

126. L-DOPA ANALOGS AS NEW ANTITUMOR AGENTS

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L-Dopa methyl ester has demonstrated significant antitumor activity in 1) leukemia, 2) P388 leukemia, 3) B-16 melanoma, and 4) C-1300 neuroblastoma. Furthermore, new analogs of L-dopa, specifically 3,4-dihydroxybenzylamide and N-acetyldopamine, exhibited enhanced antitumor activity with decreased neurotoxicity. We have proposed a mechanism of action involving inactivation of DNA polymerase by sulfhydryl group scavenging by dopa quinone. We now propose to 1) prepare analogs of dopa and dopamine in order to increase antitumor activity and diminish neurotoxicity, 2) undertake schedule dependency studies, since preliminary experiments indicate that frequent administration results in a marked enhancement of antitumor response, 3) conduct cell cycle phase sensitivity studies in order to ascertain if DNA synthesis inhibition is a primary site of action, 4) using permeabilized cells, examine directly the incorporation of thymidine triphosphate in order to define the biochemical level at which growth inhibition occurs.

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127. EXPERIMENTAL APPROACHES TO THE TREATMENT OF CNS MALIGNANCY

Poplack, D. G., Glaubiger, D. L., Gangji, D., Bleyer, W. A., Wood, J. H., Ommaya, A., Cohen, L., Cohen, S., Schwade, J., Strong, J. M., Jackson, D., Holcenberg, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Pediatric Oncology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Experimental approaches to the treatment of meningeal leukemia and other meningeal and non-meningeal CNS neoplasms are explored. A unique subhuman primate model which allows sterile, repetitive access to the cerebrospinal fluid is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities attendant upon various chemotherapeutic and radiotherapeutic treatments; and to evaluate and screen, in a preclinical setting, new CNS treatment modalities and drug schedules. Information gained from studies with this model is then applied to the design of clinical protocols used to treat patients with meningeal and non-meningeal CNS malignancies.

BIBLIOGRAPHIC REFERENCES: Gangji, D., Cohen, L., Bleyer, W.A., Vigersky, R., Glaubiger, D., and Poplack, D.G.: Methotrexate CNS Pharmacokinetics in Combination

Chemotherapy. In: Whitehouse, J. and Kay, H. (Eds.): CNS Complications of Malignant Disease, London, MacMillan Press Ltd., 1979, pp. 407-412. Jackson, D.V., Castle, M.C., Poplack, D.G., and Bender, R.A.: Pharmacokinetics of vincristine in cerebrospinal fluid of subhuman primates. Cancer Res. 40: 722-724, 1980.

128. GROWTH AND TREATMENT OF HUMAN GLIOMAS IN ATHYMIC MICE

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Our objective is to establish the value of the athymic mouse as an in vivo system for the investigation of human brain tumors. Both human glioma-derived permanent cell lines and freshly transplanted human gliomas are implanted subcutaneously into athymic mice (BALB/c background). Tumors are serially passaged in animals until growth stabilization occurs. The tumors are then exposed to a variety of chemotherapeutic agents, either singly or in combination, and the effect on tumor growth is observed. Quantitative assessments of growth patterns and treatment response as well as morphological analyses of serially passaged tumors are made, and comparison between parameters in animals and tumor behavior in the original host is attempted.

129. CHEMOTHERAPY OF MALIGNANT BRAIN TUMORS

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We have positively identified the presence of a new and novel amino acid in ribosomes.

The presence of this amino acid, gammacarboxyglutamic acid, has been correlated with the stage in the growth cycle cells. Since the most effective chemotherapeutic agents act at the level of the ribosome, we are exploring the role of warfarin in interfering with the production of this amino acid in altering growth of cells in tissue culture.

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130. POLYAMINES, POLYAMINE ANTIMETABOLITES AND CELL GROWTH

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The role of the polyamines in cell growth and replication will be investigated by following changes in intracellular levels of the polyamines and in the activities of their biosynthetic enzymes, over the course of the cell cycle. Regulation of polyamine biosynthesis will be studied using inhibitors of nucleic acid and protein synthesis. The effect of polyamine antimetabolites on cell cycle traverse will be studied also. To provide a basis for the rational design of improved polyamine antimetabolites the structural requirements for binding to the active site of ornithine decarboxylase will be investigated. These quantitative structure-activity correlations will examine inhibition of ornithine decarboxylase by a series of structural analogs of ornithine. Finally, the potential applicability of polyamine antimetabolites to the chemotherapy of intracranial tumors will be explored using in vitro and in vivo colony forming efficiency assays and increased life span studies.

131. PHARMACOLOGICAL AND CHEMOTHERAPEUTIC STUDIES OF AZQ (NSC-182986) AS A POTENTIAL CNS ANTITUMOR AGENT

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2,5-Diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone (AZQ: NSC-182986) is a new antitumor agent belonging to the chemical class of compounds known as aziridinylbenzoquinones. It shows a broad spectrum of antitumor activity in murine tumor sytems; in particular, it has been found to be active against P388, L1210 and ependymoblastoma when these tumors are inoculated intracranially. The objectives of this project are: (a) to study the disposition and metabolism of AZQ in rats and dogs; (b) to develop a suitable method for detecting and quantitating parent AZQ and its metabolites in biological fluids and tissues; and (c) to develop a tumor model system for testing AZQ and other potential antitumor agents that cross the blood-brain barrier.

132. SYNTHESIS OF POTENTIAL CNS ANTITUMOR PRODRUGS

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The synthesis of triorganosilylated carbamates and phosphoramides bearing a nitrogen mustard functionality will be continued to provide new classes of potential CNS antitumor prodrugs. Kinetics of their hydrolytic reactivity will be measured to gauge their relative 'activation' rates, i.e., desilylation leading to bis-alkylating agents. A variety of known anticancer drugs which may be converted to organosilyl derivatives will be similarly studied, and all compounds will be submitted to the National Cancer Institute for preliminary anticancer screening tests.

BIBLIOGRAPHIC REFERENCES: Synthesis, Base-Catalyzed Hydrolytic Reactivity, and Anticancer Evaluation of O-Aryl Phosphordiamidates as a Novel Class of Pro (phosphorodiamidic acid mustards), Fang-Ting Chiu, Fai-Po Tsui, and Gerald Zon, J. Med. Chem., 22, 802 (1979).

133. CLONOGENICITY AND DRUG RESPONSE OF SORTED HUMAN GLIOMA CELLS

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The objectives of this research proposal are to develop cell sorting procedures for human malignant gliomas and to initiate investigations into the clonogenicity of each population, their cell kinetics and perturbation by chemotherapeutic agents. Vital DNA staining and the aseptic sorting capability of a fluorescence activated cell sorter will allow examination of each population individually in vitro. The clonogenicity of each sorter population will be assayed via a colony formation assay and cell kinetic studies will be carried out by means of standard or double autoradiography and flow cytometric techniques.

The major goals of this project are: 1) To determine if clonogenic cells are confined to one population; 2) to understand the similarities or differences in response to drugs among these populations; and 3) to correlate the results of such studies to clinical response.

134. PROGRAM FOR TREATMENT OF MALIGNANT BRAIN TUMORS

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This continuance of a program project represents the combination of laboratory and clinical research sharply focused on the therapy of human brain tumors. Clinical research will represent Phase II studies and Phase III trials as a major chemotherapy effort for malignant gliomas. The laboratory effort is an interdisciplinary approach designed to maximize the most effective use of presently available therapeutic modalities. Included in the laboratory program are investigations involving biochemistry, biophysics, pharmacology and experimental chemotherapy.

135. SYNTHETIC HYDROPHILIC POLYMERS FOR EYE SURGERY

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The general objective is to investigate the physicochemical and physiological properties of ophthalmic materials and devices. A strong, elastic, soft hydrogel obtained by solution polymerization of methyl acrylate with 2-hydroxyethyl acrylate and simultaneous crosslinking with glycol diacrylate is being evaluated as a scleral buckling implant for use in retinal detachment surgery. A silicone rubber refillable implant for constant delivery of carmustine (BCNU) to the eye is being tested for chemotherapy of ocular melanoma. Sustained delivery of rifampin (a major antimycobacterial agent) from hydrogel contact lenses and ocular inserts is under investigation for the treatment of tuberculosis uveitis, a common disease in developing nations. Silicone rubber contact lenses offer ideal oxygen permeability for extended wear. However, certain complications, including possible epithelial damage, arise when lenses become adherent to the cornea. One of the possible causes being studied is the high water vapor permeability of silicone rubber.

BIBLIOGRAPHIC REFERENCES: Liu, H.S., Refojo, M.F., and Albert, D.M.: Experimental combined systemic and local chemotherapy for intraocular malignancy. Arch. Ophthalmol. 98:905-908, 1980. Refojo, M.F., Natchiar F., Liu, H.S., Lahav, M., and Tolentino, F.I.: New hydrophilic implant for scleral buckling. Ann. Ophthalmol. 12:88-92, 1980.

136. AN EXPERIMENTAL CANCER CHEMOTHERAPY PROGRAM

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The overall objective of this program is to improve the chemotherapy of human cancer. To accomplish that objective a coordinated series of multidisciplinary studies is conducted as follows: 1. Selected agents, synthesized within our Institute and from outside sources, are tested in established experimental tumor systems, in tumor cells in culture, and in special models for antitumor activity as well as in antiviral assays both in vitro and in mice. 2. Additional systems, new or new in our laboratory, are developed and utilized for antitumor tests, such as a rat colon carcinoma or models for metastatic brain tumors. We also investigate the action and effects of antitumor agents on immune systems. 3. To improve the usefulness of known chemotherapeutic agents we attempt the development of new combination therapy regimens, including immunopotentiators. 4. Studies of the mechanism of therapeutic action of antitumor agents are aimed at elucidating primary biochemical lesions, the nature of resistance, and the factors responsible for therapeutic selectivity. In regard to selectivity, emphasis is placed on transport mechanisms, in particular, those concerned with the uptake and retention of antifols.

137. CHEMOTHERAPY AND PERTURBED BRAIN TUMOR CELL KINETICS

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The overall objectives of this proposal are: to determine the characteristic features of brain tumor cell kinetics; to define the principles of cell population proliferative behavior of animal brain tumors in response to therapeutic agents; and, based on cell kinetic analysis, to develop treatment regimens that will improve brain tumor therapy. To accomplish these objectives 9L brain tumor cells in monolayer culture, spheroids and intracerebral 9L tumor will be employed. Perturbations induced by each chemotherapeutic agent will be analyzed by autoradiographic procedures (PLM curves, pulse or continuous labeling, double autoradiography, etc.), flow cytometry, cinephotomicrography and centrifugal elutriation. This systematic attack on the kinetics of perturbed tumor cell populations should lead to the rational development of improved therapeutic regimens for the treatment of malignant brain tumors.

BIBLIOGRAPHIC REFERENCES: Nomura, K., Hoshino, T., Knebel, K., Barker, M.: Effect of dibutyryl c-AMP on cell cycle progression of rat brain tumor cells in vitro. In Vitro 14:174-179, 1978. Hoshino, T., Barker, M., Nomura, K., Knebel, K.: DNA analysis of transplantable rat brain 9L tumors in vivo and in vitro. Proc. AACR and ASCO 17:174, 1978.

138. BIOLOGICAL, IMMUNOLOGICAL AND CHEMOTHERAPEUTIC STUDIES OF HUMAN BRAIN TUMORS

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In this study human brain tumors are evaluated in a tissue culture environment as to their basic biological behavior, their response to chemotherapeutic agents and the detailed immunological interactions between the host and the tumor. A primary goal of this work is to improve the therapy of patients by understanding the basic cellular biology of malignant human brain tumors.

BIBLIOGRAPHIC REFERENCES: Wood, W.C., Kornblith, P.L., Quindlen, E.A., and Pollock, L.A.: Detection of humoral immune response to human brain tumors. Cancer 43: 86-90, 1979. Hochberg, F.H., Linggood, R., Wolfson, L., Baker, W.H., and Kornblith, P.: Quality and duration of survival in glioblastoma multiforme. JAMA 241: 1016-1018, 1979.

139. DDMP FOR MENINGEAL LEUKEMIA (HUMAN, DOGS)

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This is part of a broader project. A summary of this subproject is not available.

140. PHARMACOKINETICS OF BRAIN TUMOR CHEMOTHERAPEUTIC AGENTS (MAMMALS, HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

141. EXPERIMENTAL CHEMOTHERAPY OF BRAIN TUMORS (MICE, RATS)

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This is part of a broader project. A summary of this subproject is not available.

142. CHEMOTHERAPY TRIALS ON TRANSPLANTED GLIOMAS IN MICE

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This is part of a broader project. A summary of this subproject is not available.

143. GROWTH KINETICS IN MURINE GLIOMAS RELATING TO DRUG DOSAGE

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This is part of a broader project. A summary of this subproject is not available.

144. UPTAKE OF DRUGS IN BRAIN TUMORS IN MICE

Shapiro, W. R., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

145. PHARMACOLOGY AND BIOCHEMISTRY OF HUMAN NEURAL CREST AND HEPATIC TUMORS (NUDE MICE)

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This is part of a broader project. A summary of this subproject is not available.

B. RADIOTHERAPY

146. PRECLINICAL RESEARCH ON THE NEGATIVE PION BEAM AT TRIUMF - PHYSICAL, IN VITRO BIOLOGICAL, AND IN VIVO BIOLOGICAL MEASUREMENTS

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OBJECTIVE: The goal of this program is to provide the technical capability as well as the necessary physical and radiobiological data to mount, at TRIUMF, an effective clinical trial of negative pi-meson beams in the treatment of cancer.

APPROACH: The program can be divided into three components: 1. Physical measurements - This includes the technical development of the facility and its operating or tuning procedures as well as the necessary dosimetry information so that one may deliver to a patient an appropriately distributed dose. 2. In vitro biological measurements -Detailed information on the RBE and OER characteristics of a variety of negative pion dose distributions is obtained using cultured cells. Both cell survival and molecular damage endpoints are being observed. 3. In vivo biological measurements - The effect of negative pion on both normal and tumor tissue is studied in mice, using a variety of endpoints: mouse skin, gut, lens of eye, spinal cord, stem cells and two transplantable tumors.

PROGRESS: Absolute dosimetric measurements have been carried out for the pion beam with an estimated accuracy of plus or minus 7%. Microdosimetry and star density measurements have been used to characterize the quality of the dose distribution.

In vitro measurements of cell inactivation have provided RBE profiles for a number of different beam tunes, and have also been used for OER determinations and for measurements of split dose recovery. In vivo experiments with mice and pigs have also begun. Mouse foot (skin) studies have used 1, 2 and 10 fraction regimes. The 10 fraction experiment shows and RBE of 1.4-1.6 for peak pions.

First patient treatment began at TRIUMF in November 1979 and involved comparative pion and X-ray treatment of multiple subcutaneous skin tumor nodules.

147. TUMOR TREATMENT

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OBJECTIVE: The improvement of the treatment of cancer through the exploitation of high energy radiation equipment and the continued development of accurate methods of radiation dosimetry and methods of dose calculation.

APPROACH: We have developed a system of dosimetry which is now in general use throughout the world. There are nevertheless many as yet unsolved problems: a) The use of CT scanners has provided an enormous increase in the detail of anatomical information that is available for making allowance for tissue inhomogeneities. We are continuing to develop methods to utilize this information profitably. This involves both experiment and the development of dose calculation methods using computers. b) High energy linear accelerators provide radiation beams which are often badly contaminated with either low energy photons or electrons. We are continuing to study these beams and trying to develop methods for improving them. c) There are still uncertainties in methods of measurement of absorbed dose from high energy radiation. We have embarked on a program to explore some new ideas that have appeared in the literature for the further development of calorimetric methods for measurement of absorbed dose. d) We are continuing to collaborate with our radiation oncologist staff in solving special treatment problems related to radiotherapy.

PROGRESS: a) Many of our past developments have been incorporated into a treatment planning computer system marketed by AECL under the name TP-11. We are currently developing the use of CT derived information with this system. b) We have devised methods of beam measurement and special filters for the improvement of high energy photon and electron beams. c) We have begun construction of a water calorimeter for the absolute measurement of dose. d) Examples of special treatments that have been devised are half-body treatments for disseminated metastases and treatment of melanoma of the optic disc.

148. INTENSIVE TREATMENT OF RETINOBLASTOMA

Schipper, J., Lagendijk, J. J., Tan, K. W., Rutgers, D. H., Academic Hospital, Dept. of Radiotherapy, *Catharijnesingel 101, Utrecht, Netherlands*

OBJECTIVE: Improvement of the results of the conservative treatment of retinoblastoma by: 1) optimalization and improvement of the irradiation techniques and 2) combination of radiotherapy with hyperthermia.

APPROACH: 1) Development of an accurate irradiation technique for retinoblastoma. 2) Evaluation of visual results and ocular complications following radiotherapy for retinoblastoma. 3) Treatment of retinoblastoma with radiotherapy combined with hyperthermia: a) development of a computer-controlled microwave heating technique for local hyperthermia of the posterior half of an affected eye; b) development of a temperature measuring technique for determination of the temperature in and around the eye without disturbing the microwave field and the heat distribution; c) determination of temperature distributions in the rabbit eye with or without microwave heating and correlation of the results with a mathematical model; d) study of the damage to the rabbit eye (particularly the lens) after radiotherapy, microwave hyperthermia, or a combination of both; e) study of the effect of radiotherapy combined (or not) with microwave hyperthermia on retinoblastoma implanted in the anterior chamber of the eye in nude mice; f) study of the effect of radiotherapy combined with hyperthermia on the cell kinetics of retinoblastoma in the above-mentioned model, with flow cytofluorometry.

149. LATE EFFECTS OF THERAPEUTIC IRRADIATION ON THE CNS

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OBJECTIVE: To find the dose-limiting factors for normal tissues in radiation therapy: the vascular system of the rat brain and its physiology were selected as the most suitable system.

APPROACH: Multidisciplinary approach: Cell kinetics: Dr. Calvo -Ulm, W. Germany; qualitative morphology: Dr. Hopewell - Oxford; angioarchitecture: Dr. Reinhold - Rijswijk; circulation physiology: Dr. Keyeux - Louvain, Belgium; ultrastructure: Dr. Maisin - Mol, Belgium; and biochemistry: Dr. Gerber - Mol, Belgium.

PROGRESS: Some contradictory findings have been encountered. Physiological changes appear to precede vascular abnormalities. The latter develop in the course of 1-2 years after irradiation. The first signs are the development of tiny teleangiectases.

150. DNA DAMAGE AND REPAIR IN IRRADIATED BRAIN AND BRAIN TUMORS

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The objective of this research is to elucidate the molecular mechanisms responsible for the differences in DNA repair kinetics observed in normal cells (brain, lymphocytes, macrophages) and tumor cells after irradiation or treatment with drugs. Our previous work indicates that 1) normal nondividing neurons repair radiation-induced DNA damage at a much slower rate than dividing brain tumor cells, 2) structural changes in the DNA of neurons can be used to evaluate the long-term effects of brain irradiation, and 3) recovery from potentially lethal damage is a major determinant of radiocurability in 9L brain tumors. We propose to use either the alkaline elution technique of Kohn or our alkaline sucrose gradient technique in slow reorienting zonal rotors to define the DNA damage and repair kinetics after treatment with radiation, drugs, or combinations of drugs with radiation in tumors and normal cells isolated from or near the tumors. The tumor model will be either intracerebral or subcutaneous 9L tumors in Fischer 344 rats. Normal cells will be 1) cerebellar neurons, or 2) lymphocytes and macrophages isolated by centrifugal elutriation from 9L tumors. The alkaline elution analysis will determine the relative amount of DNA-DNA crosslinks, DNA-protein crosslinks and DNA strand breaks in treated cells. Repair kinetics will be evaluated

by the relative rates and extent of removal of these different types of damage. Differences in the DNA repair kinetics among the various cells studied may be due to differences in the DNA template structure or differences in the availability or function of the repair enzymes. The susceptibility of chromatin isolated at various steps of the repair process to digestion by nucleases or its ability to support polymerase activity will be used to evaluate the template theory. Assays for alpha and beta polymerase activity from both normal and malignant cells will be used to test the repair enzyme theory. We will attempt to determine whether critical periods in the DNA repair process are associated with changes in either the DNA template structure or the quantity or quality of the repair enzymes.

BIBLIOGRAPHIC REFERENCES: T.S. Wang and K.T. Wheeler, Repear of X-Ray-Induced DNA Damage in Rat Cerebellar Neurons and Brain Tumor Cells. Radiat. Res. 73: 464, 1978.

151. STUDIES OF BORON NEUTRON CAPTURE THERAPY

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This proposal is an experimental plan to evaluate the therapeutic effectiveness of boron neutron capture therapy (BNCT) for the treatment of brain tumors. Brain tumor bearing neonate beagles will be treated by BNCT with a range of radiation doses delivered in a single acute irradiation. Treated animals will be sacrificed at different time periods (7, 14, 21, 45 days) post-irradiation to assess the evolvement of tumoricidal effect produced. Normal animals (neonate beagles and young monkeys) will be neutron irradiated after 10B compound infusion to observe late occurring radiation effects, especially within intracranial tissues at risk. Clinical observation of these growing animals before sacrifice will aid in the full assessment of the radiation effects, in vivo. 10B enriched Na2B12H11SH, manufactured by a U.S. company, will be the boron compound used for all these studies. Neutron induced alpha-autoradiography will be developed to measure the microscopic distribution of 10B within cells of tumor, brain and other tissues and thereby allow an accurate determination of 10B dose at the cellular level. The increase of the neutron flux distribution in tissue will be investigated by changing the therapy beam of the MITR-II, and the concomitant biological effects will be measured using the same animal model.

152. LATE EFFECTS OF FAST NEUTRONS ON CORD-BRAIN-LUNG

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This investigation was designed to study the late effects of fast neutrons (15 MeV avg.) on the brain, spinal cord or lung of dogs, irradiated with the same fractionation schedule used clinically for cancer management, compared with the late effects of megavoltage x-irradiation delivered in a clinical schedule. The late effects on the brain and spinal cord have been quantitatively evaluated by neurological exams, visual and sensory evoked responses and computerized tomography. Changes in the lung have been evaluated through pulmonary function studies, radionuclide evaluation of ventilation, perfusion, and aerosol deposition, computerized tomography and biochemical analysis of irradiated tissue.

Of the 152 dogs irradiated 78 are alive at the end of 5 years. Values of the relative biological effectiveness of fast neutrons have been obtained for the above parameters through 42 months post-irradiation. Quantitative histopathological evaluation of routine and special stains has begun. To date four tumors have been found within the irradiated fields.

153. WHOLE BRAIN IRRADIATION WITHIN THE THERAPEUTIC RANGE

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As a part of a continuing series of studies on the delayed effects of ionizing irradiation on the brain of the monkey, the current effort includes sequential observations after whole brain exposure to 6000 rads of super-voltage radiation, in divided doses over a six-week period. The protocol simulates as closely as possible that used by twelve medical centers in therapy of malignant gliomas in humans.

The current phase is primarily concerned with converting the assembled data to manuscript form for publication in scientific journals.

154. BIOPSYCHOLOGICAL STUDIES OF MICROWAVE IRRADIATION

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A study of longevity of mice that were exposed in utero to near-lethal doses of 2450-MHz microwave radiation is entering its third year and, as expected, mortality is claiming increasing numbers of control and radiated animals. Spontaneous tumors have appeared in two controls (n equals 81) but not in irradiated mice (n equals 83), a difference much too narrow to exclude chance.

Acute studies have focused on distributions of thermalized energy in the brain and lower body of anesthetized rats, guinea pigs, and rabbits during brief but near-lethal exposures in a 2450-MHz multi-path field. Pre-exposure temperatures within an animal differ anatomically by 0.5 to 1 K (e.g., colonic vs. superficial cortex); radiation-induced whole-body delta Ts of 4.5 to 12 K are associated with altered thermal distributions that differ both anatomically and among species. The only apparent rule is that of thermal privilege of the brain stem. Blood cooled in the facial sinuses by inspired air (at the ambient temperature of the laboratory) appears to be preferentially routed to the Circle of Willis during microwave heating.

Continuing studies of escape behavior by rats in intense 918-MHz multi-path fields have confirmed that radiation per se, even at levels that prove lethal within minutes, lacks the sensory saliency to promote escape learning, but that pairing of photic (and to a lesser extent, acoustic) stimuli as sensory cues with irradiation can motivate successful escape. These findings support claims by NIOSH scientists that workers exposed to intense RF fields from industrial sources experience malaise but do not attribute symptoms of illness to the stray radiation.

A total of 12 pilot studies have been completed with the aim of determining optimal conditions for adjuvant treatment by microwave hyperthermia of experimentally induced glioblastoma in rats.

155. CLINICAL STUDIES USING HYPERTHERMIA IN THE TREATMENT OF MALIGNANT GLIOMAS OF THE BRAIN

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The induction of hyperthermia in brain tumors by transcutaneous deposition of microwave energy is under investigation in this laboratory. To determine the upper limits of time and temperature for microwave irradiation without concomitant damage to nervous tissue, selective heating of the rat head was produced by shielding the remainder of the body with Eccosorb. Radiation with microwaves at 2450 MHz was delivered with a type A applicator in initial studies. The integrity of the blood-brain barrier was studied using horseradish peroxidase. Animals were injected intravenously with 1.8 mg per 10 gm body weight thirty minutes prior to sacrifice. Groups of rats were studied at brain temperatures of 40 degrees, 42 degrees or 45 degrees C. In animals with normal body temperature (37 degrees C), the blood-brain barrier could be disrupted with several combinations of brain temperature elevation and duration. Effort to duplicate these findings with a second applicator consisting of dielectric-loaded rectangular waveguide were unsuccessful. It was found that the initial rate of power depostion was lower, due to dielectric breakdown and increased power reflection. Because of the discrepancy in results obtained with these two applicators, a third type was constructed, consisting

of corrugated waveguide which was dielectric-loaded With this applicator, it was possible to disrupt blood-brain barrier integrity with the same combinations of time and temperature which had been established previously with the type A applicator. It was concluded that the high initial rate of energy deposition possible with the type A and corrugated waveguide applicators was required to disrupt blood-brain barrier integrity. These findings indicate that, in addition to the level and duration of brain temperature elevation, initial power deposition rate is important in producing barrier disruption.

156. HYPERTHERMAL RADIOTHERAPY FOR BRAIN TUMORS USING FOCUSSED MICROWAVES

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OBJECTIVE: To evaluate microwave-induced heating of specific brain regions as a possible adjunct to ionizing radiation therapy, chemotherapy, and surgery for the treatment of brain tumors. The intent is to take advantage of present capabilities in selectively heating localized regions of tissue with the intent of producing differential hyperthermal sensitization of malignant versus normal tissue; and to extend and apply these capabilities to the brain.

APPROACH: The first phase of this project is the completion of the biomedical engineering necessary for the fabrication and programming of a multiple-beam microprocessor controlled microwave diathermy. We will then test its ability to generate and control localized thermal fields in brain phantoms and in vivo in feline brain.

PROGRESS: We have already performed tests on an implantable microwave antenna and on whole-body heating.

157. FOCAL MICROWAVE RADIATION THERAPY - PRECLINICAL EVALUATION

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Focal microwave-induced hyperthermia is being studied in an attempt to evaluate its utility in the management of glioblastoma multiforme. Studies consist of a) neuroradiobiological assays of thresholds of damage to normal brain following focal heating, b) dosimetric evaluation of heating efficacy, and c) engineering developments to improve the delivery and controllability of microwave-induced hyperthermia. Two methods of heating are being employed - one invasive and one non-invasive.

158. RADIATION RESPONSE OF CULTURED HUMAN GLIOBLASTOMA CELLS

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This is part of a broader project. A summary of this subproject is not available.

C. IMMUNOTHERAPY

159. CLINICAL AND BIOLOGICAL MANIFESTATIONS OF IMMUNOTHERAPY IN CANCER EYE OF CATTLE

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OBJECTIVE: Evaluate the manifestations of immunotherapy in cattle afflicted with bovine ocular squamous cell carcinoma (cancer eye). Establish squamous cell neoplastic tissue in culture. Attempt to isolate an etiologic agent. Identify tumor-associated antigens serologically and by immuno-electron microscopy.

APPROACH: Tumor bearing animals will undergo injection either by the intramuscular route, into the bulbar conjunctiva or directly into the tumor. The separate inoculum will consist of lymphocytes from sensitized rabbits or calves, immune serum from the same animals and a phenol extract of lysed squamous cells from an active carcinoma. Serum samples will be collected routinely to be assayed by immuno-electron microscopy, immuno-fluorescence, virologic and serologic procedures. Clinical evaluation of tumors will be accomplished weekly and a permanent record will be prepared on film.

PROGRESS: Six cows each bearing histopathologically defined squamous cell carcinomas (BOSCC) were divided into 2 groups of 3 and treated individually by immunotherapy with 2 different modals. Three cows were treated with neuraminidase treated cells injected into the tumor mass at 3-week intervals for 7 months; and three cows were treated with Bacillus Calmette-Guerin (BCG) by injection of this adjuvant into the base of the tumor mass. BCG was injected during two treatment periods, with a 6-7 weeks rest separating the periods. Biopsy tissues were collected at the completion of the experiment and submitted for histopathologic examination. Blood samples were collected monthly to monitor the blastogenic response of bovine lymphocytes to pokeweed mitogen and BOSCC antigens. All tumors injected with neuraminidase treated cells grew progressively without significant microscopic changes in the malignant tissue. One tumor was treated with liquid nitrous oxide to arrest tumor growth by freezing. Although the exophetic tumor mass was reduced by necrosis, the malignant tissue invaded the orbital sinuses. One small (6 mm) tumor treated with BCG regressed after the first treatment series. This tumor was not treated during the second series and reappeared after six months. Tumors on the other two cows progressed - but at a rate slower than that of tumors treated with neuraminidase.

160. CANCER EYE (IMMUNOTHERAPY AND IMMUNOPROPHYLAXIS)

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OBJECTIVE: Evaluate immunotherapeutic agents in the prevention of bovine squamous cell carcinoma.

APPROACH: Using tissue culture techniques, explore the relationships and interactions between BCG cell wall vaccine and malignant cells in culture in an attempt to elucidate the mechanism by which the vaccine is effective. If such a mechanism if found, maneuvers to increase the efficacy of cell wall therapy by manipulation of this mechanism can be attempted.

PROGRESS: Procurement: This contract was designed to determine the efficacy of cancer immunotherapeutic agents and to contribute to the development of human clinical protocols in the treatment of malignant disease. Eighty percent of the required tumor-bearing cows have been obtained and treated with various agents. Additionally, 10 tumor-bearing horses (100% of contract requirements) have also received immunotherapy. Documentation of clinical and experimental procedures will follow for the remaining contract period. Ten additional cows will be obtained for treatment with therapeutic agents to be determined by the National Cancer Institute. Immunoprophylaxis: This contract was designed to determine the efficacy of BCG cell wall vaccine in the interruption of the transformative process of tumors from benign to malignant. The first year of this contract calls for obtaining 240 lesion-bearing animals and initiating treatment in experimental groups. To date, 95 animals have been obtained, biopsied, tagged, and identified and placed into proper experimental groups.

161. THE CENTRAL NERVOUS SYSTEM AS AN IMMUNOLOGICALLY PRIVILEGED SITE

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The objective of the research project is to determine if the immunologic privilege of the central nervous system will allow it to be more susceptible to adoptive immunotherapy than are neoplasms in other organs of the body. More specifically, we have been trying to document whether such immunotherapy using various immune populations can retard the growth of a rodent brain tumor.

The research plan that is employed has evolved over the past several years. Essentially, our protocol is based upon a comparison of the survival in control rats compared with those that have been periodically treated with injections of various types of immune cells directly into the animal's brain tumor

cavity. More specifically, adult Fischer rats were injected stereotactically with 2.5 x 10 to the third power 9L tumor cells into the right cerebrum. With this treatment regimen untreated rats die from their brain tumor at approximately 21 days after injection. The treated animals were injected with lymph node cells that have been obtained from both normal animals and animals who have been specifically immunized against the tumor being used for the challenge.

The results of some of our experiments are as follows: the most effective cell population that we have discovered to date is that obtained from animals who themselves have a brain tumor and are subsequently challenged with irradiated tumor cells in incomplete Freund's adjuvant. Such cells can be made even more effective if they are cultured in vitro for 4 days in the presence of the irradiated tumor cells.

In the months to come, we will attempt to discover ways in which such immune lymphocytes can be made to reproduce in tissue culture, so that repetitive courses of immunotherapy can be carried out. The clinical implication of this work will be direct, because patients with clinically incurable glioblastomas can in fact be treated with a similar treatment protocol if it appears promising in this experimental rat model.

162. IMMUNOTHERAPY OF EXPERIMENTAL BRAIN TUMORS

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Because of the ways in which CNS neoplasia is unique, it appeared to us that intra-tumor adoptive immunotherapy offered the most promise. In order to determine which population of immune cells would be most effective in retarding tumor growth, we carried out a series of Winn assays. In our first set of experiments, we determined that peritoneal exudate cells from animals that had been previously immunized against the specific tumor cell line could prevent the subcutaneous growth of this brain neoplasm. We next determined that such a cell population would in fact be as effective in retarding brain tumor growth when injected intracerebrally either alone or combined with systemic brain tumor chemotherapy.

Since it would appear difficult to conceive of using peritoneal exudate cells in any form in human brain tumor immunotherapy, we next evaluated the effectiveness of lymph node cells when using adoptive immunotherapy. The results of these experiments indicated that the most effective lymph node population was that which was obtained from previously immunized rats, and in which the lymph node cells were not passed over nylon wool columns. Once again, when this cell population was injected intracerebrally, we achieved the prolongation in survival in animals harboring a 9L brain tumor. In our most recent experiments, we concluded that irradiated whole tumor cells were a better mode of antigen presentation compared to either gluteraldehyde-treated tumor cells or a KCl extract of the tumor cells. In addition, other experiments indicated that peripheral lymph node cells obtained from animals who themselves have brain tumors are at least as effective as donor cells taken from animals who do not themselves harbor a brain tumor.

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163. IMMUNOTHERAPY OF INTRACRANIAL TUMORS IN MICE WITH THE USE OF IMMUNE RNA EXTRACTS

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PROGRESS REPORT: As expressed in our previous progress report, after having studied over 1,000 mice using immune RNA versus anti-tumor immune spleen cells, it was found that extracts of immune RNA were totally unsuccessful in attenuating intracranial live tumor implants; however, even in small ratios of two donor spleen cells given to one recipient statistical evidence of immunological transfer was shown. At that point the study was terminated since it was clearly evident that immune RNA extract in BALB/c mice either presents serious technical difficulties or it does not produce therapeutic effects.

During those experiments several unexpected incidents occurred in which we inadvertently provoked 'tumor enhancement' (or immunological paralysis). We then decided to use a model of experimental allergic encephalomyelitis in rats and tried to replicate that 'immunological paralysis' and 'immunological enhancement' since both effects have important clinical implications.

Up to this date our studies of immunotherapy of intracranial tumors have been presented at the North Central Mexico and Southwestern U.S.A. Neurology/Neurosurgery/Psychiatry Meeting in Tucson, Arizona, in October 1979. The results on the EAE rat model have been submitted for merit review and some of these will be prepared for publication shortly.

164. IMMUNOLOGY OF BRAIN TUMORS

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OBJECTIVES: (1) To use specific tumor antigen and lymphokine in combination with macrophages and lympocytes to develop specifically sensitized cells that effectively and specifically kill tumor cells; (2) to establish animal models to develop mechanisms to deliver specifically primed and sensitized cells; (3) to develop in vitro assays of human primary glial tumors to determine the effectiveness of autologous specifically primed and sensitized mononuclear cells.

PLAN: Autologous lymphocytes and monocytes both in man and in experimental animal (mouse glioma 261) will be primed with human derived lymphokine and crude and refined tumor antigen. These cells will be incubated with the specifically sensitized tumor cells and tumor cytotoxicity measured by inhibition of thymidine uptake and chromium release. Ultrastructural studies will be carried out to study the specific effects of lymphokine upon lymphocytes and monocytes.

METHOD: These studies utilize an automated cell harvester to measure chromium release and thymidine uptake. Morphological studies will involve light, phase and transmission electron microscopy. Effectiveness of autologous lymphocytes in the experimental model will be determined by tumor growth and survival times.

CLINICAL RELATIONSHIP: This area of research is highly relevant to the development of specific immunotherapeutic regimens for the treatment of human malignant glial brain tumors.

165. IMMUNOTHERAPY OF PRIMARY AUTOCHTHONOUS CANCER

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Mammals with naturally occurring or experimentally induced primary autochthonous cancer are being studied as guides to the immunotherapy of human cancer. It is hoped that these guides will provide information more relevant to the treatment of human cancer than that derived from studies with transplanted tumors. Cattle with ocular squamous cell carcinoma ('cancer eye') have been treated successfully by the injection of BCG cell wall vaccine directly into the tumor. Primary autochthonous tumors of mice, rats, dogs and humans are also being studied.

166. STUDIES WITH HETEROLOGOUS ANTIHUMAN NEUROBLASTOMA ANTISERUM

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OBJECTIVES: 1. To evaluate the use of heterologous anti-human neuroblastoma antiserum raised in rabbits to detect residual neuroblastoma infiltrates in bone marrow. 2. To evaluate the ability of such antiserum to kill tumor cells selectively in vitro without affecting the viability of the remaining marrow elements.

APPROACH AND PROGRESS: An antiserum has been raised in rabbits using long-term cultures of human neuroblas-

toma. The specificity of this antiserum is currently being examined. Marrow is being stored from patients with a variety of tumors including neuroblastoma. It is planned to use the stored bone marrows for autologous infusion into patients who relapse.

167. PEPTIDES AND CERTAIN ENZYMES OF VENOM

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The venoms of the rattlesnakes Crotalus cerastes cerastes, C. ruber ruber, C. adamanteus and C. molossus will be characterized for their biologically active peptides and certain proteins, using gel filtration, ion exchange chromatography, gel electrophoresis, dialysis, ultrafiltration, ultracentrifugation, and ammonium sulfate precipitation. Further chemical characterization, including amino acid analysis and sequencing, will be carried out.

The biologically active fractions will be studied, using several acute and chronic central nervous system preparations, the Bulbring nerve-muscle and crayfish DEAM preparations, neuroblastoma culture cells, tissue cultures, cardiovascular survey preparations, the rabbit ear artery-nerve preparation, vascular preparations and techniques adapted for determining the hemorrhagic and necrotizing properties of the fractions.

The data will be organized for potential use in developing an immunizing mixture of certain of the venom components for subsequent research and clinical trial. The value of the peptides medicine and as biological tools will be pursued.

D. COMBINED RADIOTHERAPY AND CHEMOTHERAPY AND OTHER TREATMENT MODALITIES

168. COMBINED EFFECTS OF RADIATION AND CYTOTOXIC DRUGS ON THE RADIATION TOLERANCE OF THE CENTRAL NERVOUS SYSTEM

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OBJECTIVE: Chemotherapeutic agents, in particular methotrexate, are currently being used in conjunction with brain irradiation as part of the prophylactic treatment of children with leukemia. It is proposed to examine the possible effects of various drug combinations on the radiation tolerance of the central nervous system.

APPROACH: It is proposed to investigate the mechanisms responsible for the development of damage in the brain and spinal cord in experimental animals. Effects will be evaluated by the quantitative assessment of cellular changes in histological material obtained by the serial killing of treated animals. The importance of field size will be investigated.

Modifications in normal tissue and tolerance produced by radiation of the spinal cord in combination with various dose levels of intrathecal methotrexate and other drugs will be determined by establishing the proportion of animals developing paralysis after 6 months. Iso-effect doses will be compared to determine the relative changes in cord tolerance.

In the brain, radiation depletion of the cells in the subependymal plate region will be used as a measure of radiation effects. Modifications in this response, produced by drug combinations, will be evaluated.

169. THE INFLUENCE OF PHYSICAL AND CHEMICAL FACTORS ON GROWTH AND PROLIFERATION OF CULTURED HUMAN CELLS

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OBJECTIVE: Studies of the influence of radiation and cytotoxic drugs on monolayer, suspension and spheroid cultured human cells.

APPROACH: The survival of human glia, glioma, osteosarcoma and thyroid carcinoma cells is studied after radiation and other disturbances by using a newly developed cloning technique. This method allows cells with a low plating efficiency to be cloned. Radiation effects on spheroids of human origin are studied by using the so-called mini-dish technique. The radiosensitivity of suspension cultured histiocytic and lymphocytic lymphoma cells has been compared by using the growth extrapolation method.

PROGRESS: A) We found that human glioma cells in culture generally seem to be much more radioresistant than the glia cells (and in fact more resistant than most other cells tested in culture). B) We found a general difference between spheroids of humans and those of rodent origin. The former had a thicker viable layer outside the necrotic central zone than the latter ones. We also found, by using pO2 microelectrodes, that the oxygen gradients had different shapes in different types of spheroids. We are presently comparing radiosensitivities of such spheroids. C) We have found that histiocytic lymphoma cells seem to be more radioresistant than lymphocytic lymphoma cells. This also correlates with clinical findings.

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170. EFFECTS OF CHEMOTHERAPY ON THE RADIATION TOLERANCE OF THE RAT CENTRAL NERVOUS SYSTEM

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OBJECTIVE: Evaluation of risk of development of late damage in the central nervous system after combined treatment with chemotherapy and radiation. For methotrexate and cytosine arabinoside, intrethecal and intravenous administrations will be compared.

APPROACH: The rat cervical spinal cord is irradiated with single and fractionated doses of 300 kV X-rays and a chemotherapeutic agent is administered shortly before irradiation. Various syndromes of radiation myelopathy are differentiated on the basis of neurological and histopathological characteristics. Tolerance dose levels are established for the various syndromes. The following drugs have been used: methotrexate, cytosine arabinoside, misonidazole and actinomycin D.

171. RADIATION ENHANCEMENT IN NERVOUS SYSTEM TUMOR MODELS

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Our objective is the utilization of chemical agents for enhancement of effect of x-irradiation on central nervous system tumors without an increase in effect on normal brain. The approach utilizes two methods of study, an in vivo system of intracerebral inoculation with survival time and tumor size as end points, an an in vitro system in which cell lethality is assessed by colony forming efficiency. Tumor cells for both models include a series of cell lines and clones derived from representative types of nitrosourea-induced tumors of nervous system in rats. Improvements in cell implantation techniques, including use of stereotaxic procedures, an agar preparation in the cell suspension, and a shift to the caudate nucleus as a target site, resulted in a model much superior to the previous freehand system and quite suitable for therapeutic studies. Studies with a number of chemical agents have shown marked differences in response among cell lines representing different types of nervous system tumors. Completion of the retinol studies has shown significant radiation enhancement on spongioblastoma and astrocytoma, but not on Schwannoma or a well differentiated glioma clone. Six-aminonicotinamide and diamide had enhancing effects on radiation in vitro and also show some evidence of enhancement in vivo when given intraperiotoneally; however, the latter effect may be obscured by toxic synergism. Serious neurotoxicity has been somewhat ameliorated by intracerebral administration. Both metro- and misonidazole have shown surprisingly little effect on either spongioblastoma or astrocytoma. BCNU has shown positive, but variable, effects on spongioblastoma, astrocytoma and Schwannoma. Studies on variation in x-ray fractionation and dosage together with multiple treatments with BCNU have indicated a marked increase in survival in compari-

son with previous single dosage and time relationships. This offers considerable promise for further exploration in other cell lines. A new agent, ACNU, has shown a significant radiation enhancing effect on a glioma clone. Contrary to others, we do not find significant direct increase in survival with ACNU alone. These studies emphasize the complexity of experimental tumor therapy in this group of tumors. Work in progress or to be initiated includes the in vitro studies with metronidazole and misonidazole as well as with retinol and other analogs. (Text Abridged.)

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172. THE UNIVERSITY OF CHICAGO CANCER RESEARCH CENTER (UCCRC) - LYMPHOMALEUKEMIA CLINICAL RESEARCH PROGRAM

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The University of Chicago Cancer Research Center: Clinical Cancer Research Program is a multidisciplinary research effort encompassing diagnosis, staging, treatment, and correlations with basic science investigations in the following broad areas: (1) Breast Cancer -Pathologic evaluation of cases and correlation with estrogen receptors, lymph node morphology, and endocrine data will be continued; antibodies to receptor protein have been prepared and efforts to develop a radioimmunoassay will continue. (2) Gastrointestinal Cancer - The program to assess vulnerability of ulcerative colitis patients to cancer continues employing histologic and cytologic assessment with colonoscopy as well as studies of humoral and cell-mediated immunity. (3) Thyroid Cancer - Comparison of irradiated patients and patients who had thyroid carcinoma without history of irradiation continues. The immunologic status of thyroid cancer patients is being evaluated. TSH receptors on the surface of thyroid tumors will be studied. Immune complexes are being determined in the circulation of the patients. (4) Comprehensive Approach to Glioma Therapy - Kirsten virus induced brain tumors in rats are employed to study pathogenesis and test radio/chemotherapeutic interventions. A study of combined surgery, radiotherapy and chemotherapy with BCNU and epipodophyllotoxin is being completed. Glia maturation factor has been purified to homogeneity and specific antiserum is being obtained. (5) Lymphoma-Leukemia Program - Sensitive cellular assays measuring nanogram quantities of Ig have been developed to study B-cell function; T-cell cytotoxicity can also be assayed accurately. These techniques are used to study neoplastic lymphocytes. The project encompasses a determination of surface T- and B-cell markers, a cytogenetic analysis, the measurement and functional role of autologous serum immune complexes, and the determination of the effects of alpha-fetoprotein, an oncofetal protein, on the neoplastic cells contained in these separate lymphocytic malignancies.

173. RADIATION ENHANCEMENT IN NERVOUS SYSTEM TUMOR MODELS

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OBJECTIVE: Achievement of chemical enhancement of radiation injury to tumors of the nervous system.

APPROACH: A series of agents will be tested in experimental models currently operational. Groups of chemicals to be investigated are chosen on the basis of known features of tumors. These agents will include lysosomal labilizers and membrane alterating compounds (vitamin A, Filipin), high electron affinity compounds (nitrohetrocyclic compounds), agents decreasing reduced glutathione (diamide and also nitrofurans), and pentose pathway inhibitor (6-aminonicotinamide).

174. SEQUENTIAL CHEMOTHERAPY AND HYPERTHERMIA IN THE TREATMENT OF EXPERIMENTAL GLIOMAS

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The effects of hyperthermia were studied in cell cultures of malignant gliomas. Explant cultures were established from two murine gliomas, an ependymoblastoma of C57BL/6 mice and a glioblastoma with intermingled sarcomatous elements of C3H mice, both induced with chemical carcinogens implanted in the brain. Cell cultures were maintained in a minimal essential medium. Hyperthermia was induced with a water bath or with microwave radiation at 2,450 MHz. Viability assays with trypan blue and fluorescein diacetate were carried out before and after heating cultures to 40, 42 or 45 degrees C for increasing lengths of time. Cell survival was related both to the level of temperature elevation and to the length of treatment. Cells of the ependymoblastoma were more heat-sensitive than those of the glioblastoma-sarcoma. Cultures of ependymoblastoma were slightly less heat-sensitive after the fourth explant generation. When DNA histograms were prepared from heat-treated glioma cells allowed to survive for periods up to 48 hours following treatment with hyperthermia, resumption of DNA synthesis was observed following an initial heat-induced impairment. Studies combining hyperthermia with individual antiglioma chemotherapeutic agents have been initiated.

175. INTRAOCULAR TUMOR VAPORIZATION BY CO2 LASER RADIATION

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Current techniques for treating intraocular tumors unavoidably damage surrounding healthy eye tissue with equivocal destruction of the neoplasm. This can cause severe visual loss or the enucleation of several thousand eyes yearly in the United States. With malignant tumors, trauma during treatment has been implicated in the metatastic seeding to other parts of the body.

The purpose of the proposed project is to develop a carbon dioxide laser vaporization system that will safely and effectively obliterate retinal and choroidal intraocular tumors with excellent hemostasis and total vaporization of the tumor tissue. Healthy ocular tissue will be spared, to better preserve vision, and to obviate the need for enucleation. In cases of malignant tumors, it is projected that CO2 laser vaporization will prove to be less traumatic and provide a longer patient survival rate than current techniques.

We have developed a delivery system to channel the carbon dioxide laser radiation to tumor masses within the eye. Laser power will be accurately monitored and specialized safety interlocks will be inclued. A flow nitrogen system will perfuse the vitreous cavity during laser treatment, allowing rapid removal of vaporized tumor tissue. The entire CO2 laser system is under construction at S.R.I. International in Palo Alto, CA, with projected delivery in December 1978.

Animal histological studies will be performed initially, to quantitate exactly the tissue effect of various carbon dioxide laser radiation modes in specific areas of the eye. When the precise parameters for safe, effective CO2 laser radiation have been established, the clinical phase will begin. Extraocular and intraocular tumors will be vaporized, with emphasis on neoplasms. The results will be evaluated through detailed biostatistical analysis. Patients will be followed for a minimum of five years.

176. OCULAR TISSUE EFFECTS OF CO2 LASER ENERGY

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Advanced diabetic retinopathy and retinal detachment are two of the leading causes of blindness. Once massive vitreous hemorrhage develops from these conditions, vision is lost and surgical repair is difficult. A frequent complication is the formation of adhesions and traction bands between the vitreous

humor and retina which produce an immobile, functionless retina. Vitreous surgery is in the early stages of development and new techniques are constantly being evaluated. The purpose of this research is to develop vitreous instrumentation which will permit the opthalmic surgeon to: (1) Introduce a CO2 laser beam (as part of the vitreous probe) directly into the vitreous cavity utilizing fiber optic techniques. This will accomplish photo-transection (cutting) of vitreoretinal adhesions as well as photocoagulation (cauterization) of neovascular vessels if bleeding occurs. (2) Utilize fiber optic techniques (as part of the vitreous probe) to simultaneously illuminate and view the vitreous cavity directly from within the eye and display the image via a video monitor. (3) Include, as part of the same vitreous probe, a system for suction and removal of vitreous hemorrhage, and reconstitution of intraocular pressure with clear vitreous substitutes. (4) Combine laser photo-transection; laser photocoagulation; fiber optic illumination and viewing; and suction and irrigation into a single miniaturized multi-function probe. (5) Test the prototype instrument, using eyes of animals, to accomplish vitreous transplantation, full thickness sclera-chorioretinal wall resection for small intraocular tumors, and transvitreal chorioretinal biopsy.

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177. MECHANISM OF ACTION POTENTIAL NA ION IONOPHORE

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We will investigate the molecular mechanism of the action potential Na(plus) ionophore by selection of mutant mouse neuroblastoma clones with defective ionophores. Clones of mouse neuroblastoma represent a homogeneous population of cells with excitable membranes. The Na(plus) component of the action potential is apparently identical to that in nerve. There are three components to the Na ion ionophore as defined by the effect of three classes of neurotoxins on the Na ion current. Tetrodotoxin and saxitoxin directly block the channel; excitatory alkaloids such as veratridine, batrachotoxin, and aconitine bind on a separate component and cause activation of the ionophore; and scorpion toxin acts on a third component by preventing inactivation of ionophore. We will select clones with altered Na(plus) ionophores by growing cells in the presence of one of the excitatory alkaloids or a combination of one of these alkaloids and scorpion toxin and with or without treatment with mutagens. Preliminary results indicate that less than one in a thousand cells survives in these conditions. Resistant cells would have ionophores containing components that are either altered in their response to the selecting toxin or missing altogether. We will examine mutants for binding of radioactively labelled toxins and for electrophysiological characteristics. We will be able to tell whether each of these components is found on a separate polypeptide. Also, we will be able to examine in detail the function of each component and its interaction with the other components of the action potential Na(plus) ionophore.

178. EXPERIMENTAL NERVOUS SYSTEM METASTASES - PATHOGENESIS AND TREATMENT (RATS)

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This is part of a broader project. A summary of this subproject is not available.

IV. CLINICAL THERAPY OF NERVOUS SYSTEM TUMORS

A. SINGLE MODALITY THERAPY

1. CHEMOTHERAPY

179. PROTOCOL OF TREATMENT FOR PRIMITIVE TUMORS OF THE CENTRAL NERVOUS SYSTEM WITH BCNU (PHASE III)

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CURRENT STATUS: In January 1978, the U.S. National Cancer Institute ceased providing BCNU. At that time, the study was closed to new patients. Follow-up continues for the groups of patients listed below:

Control Group: Four patients alive with survival of 7 to 24 months. Two patients have died at 8 and 9 months, respectively.

BCNU Group: a) Patient who, after 3 treatment series, interrupted treatment due to pregnancy and then continued with the 4th and last treatment. The patient is without evidence of recurrence 19 months after randomization. b) Patient who, after 3 treatment series, was scheduled to undergo surgery for osteitis of the flap. The patient then abandoned chemotherapy and is alive at 20 months without signs of recurrence. c) Patient who received 5 treatment series of BCNU. Treatment was suspended due to lack of drug. Twenty months after randomization, the patient is without evidence of recurrence. d) Patient who died after 4 treatment series due to tumoral recurrence.

BCNU-VCR Group: a) Patient who abandoned treatment after one series. b) Patient who completed the entire treatment plan. Within 24 months of evolution, there is slow progressive evolution of the glioma of the chiasma opticum. c) Patient who received 2 treatment series, then was suspended from treatment due to lack of drug. Patient is currently included in the VM 26 protocol, with 13 months of evolution.

180. AUTOLOGOUS BONE MARROW RESCUE IN LYMPHOMA AND NEUROBLASTOMA

Ekert, H., Ellis, W. M., Waters, K. D., Royal Childrens Hospital, Dept. of Clinical Haematology & Oncology, *Flemington Rd., Parkville, Victoria 3052, Australia*

OBJECTIVE: To determine whether autologous marrow rescue can be used in the treatment of Stage III and IV malignant lymphomas and neuroblastoma.

PROJECT: Intensive chemotherapy will be used to produce remission and bone marrow will be cryopreserved when remission has been induced. Where bone marrow is free of tumor, it will be harvested prior to commencing chemotherapy. Autologous bone marrow rescue will commence 36 hours after intensive chemotherapy has been administered.

PRESENT STATUS: Project at initiation.

181. ADRIAMYCIN PHARMACOKINETICS IN CHILDREN

Evans, W. E., Pratt, C. B., Hayes, A., George, S. L., Beck, W. T., St. Jude Childrens Research Hospital, Clinical Pharmacokinetics Laboratory, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee*, 38101, United States of America

The principal goals of the proposed research are to determine the pharmacokinetic parameters of adriamycin disposition in children with cancer and the relationship of drug disposition to tumor response. Among specific questions that will be asked are the following: (1) Are there significant interpatient and intrapatient differences in the disposition of adriamycin in children? (2) Do the rate of drug metabolism and the extent of drug distribution significantly influence tumor response in children with neuroblastoma? (3) Does patient age, sex, disease status, concurrent drug therapy and/or nutritional status have a predictable influence on adriamycin disposition in children with cancer?

The pharmacokinetics of adriamycin and metabolites will be determined from plasma and urine concentrations determined by HPLC-fluorescence and characterized by a multicompartment physiologic pharmacokinetic model. Distribution characteristics will be further evaluated from serial bone marrow samples obtained before and after adriamycin administration. Tumor response will be measured by changes in the cell kinetics (La-

beling Index, Mitotic Index) and flow cytometry of tumor cells obtained from serial bone marrow samples before and after adriamycin administration in children with disseminated neuroblastoma and acute non-lymphocytic leukemia. Previous studies have established the correlation between cell kinetic changes and clinical response in neuroblastoma. Patient variables such as age, sex, concurrent drug therapy, disease status and nutritional status will also be evaluated for their influence on adriamycin disposition.

The above study will provide information on the influence of adriamycin metabolism and/or distribution on tumor response in children with neuroblastoma. It may also provide us with a more rational basis for dosage escalation or chemotherapeutic changes in children that fail to respond to current therapy.

182. CLINICAL PHARMACOLOGY OF INTRATHECAL METHOTREXATE

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The goal of this project is to improve the therapeutic index of intrathecal methotrexate for the treatment of brain tumors, meningeal leukemia, and other neoplasms primary or metastatic to the central nervous system. The approaches that will be taken include 1) correlation of cerebrospinal-fluid antifolate concentrations with therapeutic and toxic effects, 2) identification of patient characteristics that influence the central-nervous-system pharmacokinetics of methotrexate, 3) Phase 2 and Phase 3 trials of intrathecal aminopterin, 4) pharmacological evaluation of the hyperbaric injection technic, 5) additional clinical experience with intraventricular chemotherapy via the Ommaya reservoir, and 6) the testing of new methods of central-nervous-system chemotherapy in murine and primate models. The latter will include evaluation of the effects of a variety of conditions on cerebrospinal-fluid methotrexate concentration: intravenous infusions of high-dose methotrexate, cranial irradiation, kystemic and intrathecal probenecid administration, body position and hyperbaric (D10W-Trendelenberg) intralumbar chemotherapy. A primate model of methotrexate-induced leukoencephalopathy will be evaluated in order to determine what factors contribute to, prevent, or reverse the toxicity.

The ultimate objective is to eliminate the need for intrathecal methotrexate. This possibility will be explored by infusing very high doses of methotrexate, 33.6 g/m2, intravenously for 24 hour periods, followed by citrovorum rescue. A prospective randomized trial is planned in patients scheduled to receive treatment for prevention of CNS leukemia.

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183. PHARMACOKINETICS OF CANCER CHEMOTHERAPEUTIC AGENTS

Mehta, B. M., Hutchison, D. J., Rosen, G., Shapiro, W. R., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

Distribution of methotrexate (MTX), citrovorum factor (CF), and 5-methyltetrahydrofolate (5-MTHFA) will be followed in serum and cerebrospinal fluid of patients with osteogenic sarcoma and meningeal carcinomatosis, using microbiological as well as other assay methods, viz. high pressure liquid chromatography, radioimmunoassay, 'Emit-and' enzyme immunoassay, and enzymic assay methods, developed in our laboratory. We propose to establish guidelines for toxicity in relation to serum concentrations of MTX, CF and 5-MTHFA, as well as to determine the effectiveness of the therapy protocol.

Distribution kinetics of MTX, CF and 5-MTHFA will also be followed in brain tumor models in rats and monkeys established by Dr. Shapiro and associates.

We also propose to isolate and characterize antibiotic resistant strains of organisms used for the assay of MTX, CF, 5-MTHFA and examine these strains for the physiological alterations associated with the newly acquired resistance.

BIBLIOGRAPHIC REFERENCES: Mehta, B.M., Shapiro, W.R., Rosen, G., and Hutchison, D.J. Distribution of folates following methotrexate-leucovorin rescue regimen in cancer patients. In: Chemistry and Biology of Pteridines, vol. 4. Kisliuk, R.L. and Brown, G.M., eds., 1979, Elsevier/North-Holland, New York, pp. 677-682. Allen, J.C., Hasegawa, H., Mehta, B.M., Shapiro, W.R., and Posner, J.B. CNS penetration of methotrexate is enhanced by hyperosmolar intracarotid mannitol and meningeal carcinomatosis. Neurology 28:351, 1978.

184. STUDIES OF REVERSIBLE OSMOTIC BLOOD-BRAIN BARRIER DISRUPTION AS A NEW MEANS OF INCREASING CHEMOTHERAPY TO THE CNS (ABBREV)

Neuwelt, E. A., Moore, R. J., University of Texas, Health Science Center at Dallas, Southwestern Medical School, Dept. of Surgery, *5323 Harry Hines Blvd., Dallas, Texas*, 75230, United States of America

Several types of metastatic malignant tumors demonstrate responsiveness to chemotherapy; however, these tumors when metastatic to the CNS show remarkable therapeutic resistance. A major reason for such resistance appears to be the existence of the blood-brain barrier (BBB) and its effect on the delivery of chemotherapy. The importance of drug delivery with regard to primary brain tumors (gliomas) is less clear.

Reversible osmotic BBB disruption has been shown to be relatively non-toxic in several species (rodent, canine and primates). When the BBB distribution is combined with intracarotid infusion of methotrexate, the tissue concentrations in the brain are significantly increased relative to those in the absence of BBB disruption.

The proposed studies will evaluate in human brain tumor subjects the effects of BBB disruption, which we have carried out on 15 occasions in patients without complications. The toxicity of barrier disruption will be studied in detail in at least 20 patients. Enhanced computerized tomography (CT) documents BBB disruption, and the present studies will explore simplified methods of evaluating the adequacy of disruption. Preliminary data suggest that simple radionuclide imaging also may provide a reliable criterion of disruption. We are now evaluating the effectiveness and/or toxicity of this enhanced mode of delivery of methotrexate to malignant CNS tumors.

Parallel animal experiments proposed include: a) evaluation of improved methods of assessing BBB disruption which are both non-invasive and invasive, b) assessment of the toxicity of intracarotid and intravenous adriamycin after osmotic BBB disruption, c) measurement of tissue levels of adriamycin by radioimmunoassay and correlation of tissue concentrations with parameters of toxicity, and d) determination of the effect of dexamethasone therapy on the sensitivity of the BBB to disruption by mannitol.

Finally, proposed studies will explore the role of the BBB in two animal models of CNS neoplasms, primary canine and rodent gliomas induced by intracerebral inoculation with the Schmidt-Ruppin strain of the Rous sarcoma virus and the Walker 256 carcinoma metastatic to the CNS in rats.

The overall objectives of our program are to explore ways to improve the conventional modes of treatment for tumors of the CNS and to utilize two different models to evaluate the osmotic BBB disruption procedure. (Text Abridged.)

185. CYTOKINETIC MODIFICATIONS OF NORMAL AND NEOPLASTIC TISSUE

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We evaluated antiguanosine (AG) immunoperoxidase staining in CHO cells in culture after mitotic selection and found previously described nuclear inclusions which correlate with S-phase activity by FMF and H2-TdR labeling. Additionally, we found diffuse nuclear staining patterns that appear to define late S, G2, M and G1 phases. We plan further studies to define sites of AG binding producing these patterns and we plan to extend this technique to other cell lines and clinical specimens. Confirmation of our findings represents a breakthrough in methodology which allows rapid determination of cell cycle phase distributions of a variety of cells and tissues. We studied hydroxyurea (HU) infusions in cancer patients and found that a bolus of 450 mg/m2 followed by 2.0-2.5 mg/m2/min x 72 hours produces and maintains serum HU levels 3-5 x 10 to the minus 4th power

and partial synchronization of bone marrow without significant toxicity (mean pre- and post-HU labeling indices 16% and 33%, medial WBC and platelet nadirs greater than 4000/mm3 and greater than 200,000/mm3). One patient's cancer (adeno, lung) responded similary. We are studying HU as above followed by MTX and 5-FU at time of peak tumor S-phase activity with subsequent VCR and BLEO at time of peak tumor G2 distribution. Higher doses of HU (550 mg/m2 bolus, 3.0-3.5 mg/m2/min x 72 hrs) produce serum HU levels greater than 10 to the minus 3rd power M, are myelotoxic and produce profound depression of marrow and tumor S-phase activity. Such doses could be used for a Phase II study of infusion of HU as a single agent. One patient with brain cancer has responded for 14 plus months.

186. BLOOD-BRAIN BARRIER DISRUPTION WITH MANNITOL IN PATIENTS WITH METASTATIC CNS TUMORS RECEIVING INTRA-ARTERIAL METHOTREXATE - PHASE I STUDY

Neuwelt, E. A., Frenkel, E., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Neurosurgery Section, *4500 S. Lancaster Rd., Dallas, Texas*, 75216, United States of America

Reversible transient osmotic blood brain barrier disruption was employed to increase drug delivery to the brain. Methotrexate was administered 33 times to six patients with brain tumors after barrier disruption. No permanent complications were seen. Serial enhanced computerized tomographic scans and quantification by computerized tomographic number indicated that disruption increased drug delivery to the tumor as well as immediate surrounding brain. Neuroradiologic evaluation demonstrated drug in the tumor persisted longer after barrier disruption than when delivered without disruption. The concentration of methotrexate in spinal fluid did not correlate with the degree of barrier disruption measured by computerized tomographic and radionuclide scans. In one patient an anatomic variation in the circle of Willis resulted in barrier disruption extending into the posterior fossa without ill effect. Osmotic blood brain barrier disruption appears to be a safe procedure in man, capable of increasing drug delivery to both malignant brain tumors and surrounding brain parenchyma.

187. PHASE II TRIAL OF AZQ IN PATIENTS WITH MALIGNANT GLIOMA AND METASTATIC BRAIN TUMORS

Ihde, D. C., Schilsky, R., Smith, B., Kornblith, P., Kelley, J., Ozols, R., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *50 Irving St. N.W., Washington, District of Columbia*, 20422, United States of America

AZQ (aziridinylbenzoquinone) is an antitumor agent specifically synthesized with sufficient lipid solubility and low ionization properties to allow ready penetration into the central nervous system. Intraperitoneal injection of the drug can cure intracerebrally inoculated tumor in some animal tumor systems. A Phase I study nearing completion has shown that the dose-limiting toxicity of AZQ in human patients is myelosuppression, with slighly greater effects on the platelet than on the leukocyte count. Mild nausea and vomiting and alopecia were also noted. The present Phase II study proposes treatment of patients with primary and metastatic brain tumors with AZQ, in an effort to document anti-tumor effects. All patients must have evaluable disease on head CT scan and must have failed brain irradiation or be asymptomatic with respect to their brain tumors. The dose will be 20 mg/m2 IV on Days 1 and 8 every 4 weeks, with dose reduction for heavily pretreated patients.

188. COMPARISON BETWEEN CHLOROZOTOCIN, TRIAZINATE, CIS-DIAMMINEDICHLOROPLATINUM (II), AND BCNU AND 5-FU (ABBREV)

Cashion, E. L., Robertson, J. T., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Neurosurgical Section, *1030 Jefferson Ave., Memphis, Tennessee*, 38104, United States of America

Since the inception of this protocol four VA patients have been treated. One patient received chlorozotocin. Two patients received triazinate and one patient received the combination of BCNU plus 5-FU. The patients on triazinate tolerated their treatments well with no side effects. The other two patients had mild nausea and vomiting following drug administration which cleared within twelve hours. The patient receiving BCNU had very mild myelosuppression, but only with his first course of treatment. Two patients have expired from tumor progression. Treatment has been discontinued on the patient receiving BCNU plus 5-FU because of clinical deterioration. The other patient continues to receive his treatments and is stable.

One autopsy has been performed.

189. THE STUDY OF INTENSIVE CHEMOTHERAPY AS AN ADJUNCT TO SURGERY AND RADIOTHERAPY FOR MALIGNANT GLIOMAS

Weiner, R. S., Day, A., Noyes, W. D., Kitchens, C. S., Ross, W., Streiff, R. R., Kramer, B., Gefter, J., Ballinger, W., Thar, T. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *Gainesville, Florida*, 32601, United States of America

The design of this study is to determine the therapeutic effectiveness of nitrosourea when dose and schedule can be maintained.

The objectives are: To determine whether the infusion of autologous stem cells following nitrosourea chemotherapy can reduce the degree and duration of myelosuppression, and to determine the hematologic and immunologic responses in patients receiving nitrosureas with and without autologous stem cell reinfusion.

190. CHEMOTHERAPY FOR MEDULLOBLASTOMA

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This is part of a broader project. A summary of this subproject is not available.

191. L-DOPA ANALOGS AS NEW SELECTIVE ANTITUMOR AGENTS FOR MELANOMA AND NEUROBLASTOMA

Wick, M. M., Sidney Farber Cancer Center Inc., Division of Clinical Pharmacology, *44 Binney St., Boston, Massachusetts*, 02115, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

192. HIGH DOSAGE METHOTREXATE AND C. RESCUE FOR OSTEOGENIC SARCOMA AND PONTINE GLIOMAS

Fletcher, W. S., Oregon State Higher Education System, University of Oregon Health Sciences Center, School of Medicine, Dept. of Surgery, *3181 S.W. Sam Jackson Park Rd., Portland, Oregon*, 97201, United States of America

This is part of a broader project. A summary of this subproject is not available.

193. THERAPY OF CHONDROSARCOMA AND CHORDOMA WITH S-35 (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

194. ACTION OF MTX IN NEUROLOGICAL SYSTEMS

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This is part of a broader project. A summary of this subproject is not available.

2. RADIOTHERAPY

195. ADVANCED BREAST CANCER - A RETROSPECTIVE ANALYSIS OF PATIENTS DEVELOPING CEREBRAL METASTASES

Sparrow, G. E., Rubens, R. D., Imperial Cancer Research Fund, Breast Cancer Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Cerebral metastases usually produce distressing symptoms and an early death. It is not known which groups of patients are most at risk from developing this complication nor its optimal treatment. Radiotherapy is the treatment used most often but although prolonged responses are occasionally seen, it often fails to palliate symptoms and may cause considerable disturbance to

the patient. Forty-two cases of cerebral metastases have been studied to analyze the results of treatment and the relevance of certain prognostic variables.

196. FAST NEUTRON BEAM RADIOTHERAPY

Griffin, T. W., University of Washington, School of Medicine, Dept. of Radiology, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

Since 6-1-71, the National Cancer Institute has supported a study of fast neutron beam radiation therapy of cancers in humans by the faculty and staff of the University of Washington. During the first 2 years, a medical treatment beam was developed at the University of Washington cyclotron by the staffs of the Division of Medical Radiation Physics and the cyclotron group. This beam, using 21.5 MeV deuterons to strike a beryllium target of 'intermediate' thickness (70% of incident deutrons absorbed), has depth dose characteristics comparable to 60Co teletherapy and an output of about 40 rad per minute at 125 cm from the target. The collimation developed for the fixed horizontal beam allows patient application within standards of safety used in conventional photon radiation therapy.

During the second 3-year grant period, the physics staff continued to refine theoretical and clinically oriented dosimetry. A computerized treatment control system was developed and made operational. Custom-made patient holding devices, i.e., chair, table and kneeling bench, were developed.

Prior to clinical application, the fast neutron beam was biologically characterized by the staffs of the Division of Radiation Biology and the Experimental Radiation Oncology Section. Acute and long-term RBEs were determined for several tissues. OERs were determined at several sites in an absorber. Animal tumor systems were developed for use in determining therapeutic ratios and treatment patterns.

Patient treatment started 9-10-73. Phase I studies of the treatment of patients with glioblastoma multiforme and extensive metastases to the neck were adequately completed for publication. Protocols were developed in cooperation with the staffs of the M.D. Anderson-TAMVEC and MANTA for glioblastoma multiforme and epidermoid carcinomas of the oral cavity.

197. FERMILAB CANCER THERAPY FACILITY

Cohen, L., Awschalom, M., Hendrickson, F., Rosenberg, I., Hrejsa, A., Kaul, R., Mansell, J., Zimmer, D., Bennett, B., Pientak, B., Universities Research Assn. Inc., *2100 Pennsylvania Ave. N.W., Washington, District of Columbia*, 20037, United States of America

The overall objective of the study is to continue a major research program for clinical trials in fast neutron therapy in order to evaluate the effectiveness of neutron therapy relative to other forms of conventional radiation therapy. Since all essential preclinical physical measurements and calibration, radiobiological studies, and pilot studies on patients referred with locally advanced disease have been completed satisfactorily, our objective at this stage is a strictly controlled experiment within a cooperative national protocol to evaluate one modality against another in randomly selected groups of patients. This work will concentrate mainly on subjects eligible for entry into the RTOG protocols: Resectable and Nonresectable Late Stage Cancer of the Head and Neck, Malignant Brain Tumors, Cancer of the Prostate and Bladder, Late Stage Carcinoma of the Cervix, Carcinoma of the Esophagus, Small Cell Carcinoma of the Lung, Carcinoma of the Pancreas, Tumors of Salivary Glands, Radioresistant Adenocarcinomas, Bone and Soft Tissue Sarcomas.

198. MANTA NEUTRON CLINICAL TRIAL FOR LOCALIZED CANCERS

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Studies are currently in progress to determine whether or not fast neutron therapy is more effective than conventional forms of treatment in controlling locally advanced malignant tumors. Controlled clinical trials have been initiated by the Middle Atlantic Neutron Therapy Association for squamous cell carcinomas of the upper aerodigestive tract and for glioblastoma multiforme tumors. Similar trials for the treatment of locally advanced tumors of the uterine cervix, urinary bladder, and prostate are to be initiated. Pilot studies for the treatment of carcinoma of the esophagus, carcinoma of the pancreas, chondrosarcomas and other locally advanced tumors are also in progress.

199. PROTON RADIATION THERAPY RESEARCH

Suit, H. D., Goitein, M., Munzenrider, J., Wang, C. C., Shipley, W. U., Gunderson, L. L., Choi, N. C., Epp, E. R., Verhey, L., Friedberg, C., Urano, M., Massachusetts General Hospital, Dept. of Radiation Medicine, *32 Fruit St., Boston, Massachusetts*, 02114, United States of America

The clinical activities of this program will be expanded further with the addition of another physician and of technical support personnel. Specific attention will be directed to treatment of patients with carcinoma of oral cavity and oro-pharynx (4 patients have been treated with floor of mouth cancers). We will initiate a prospective randomized trial of photons plus perineal boost proton vs photon alone in the treatment of prostate cancer. Also the trial of 7000 vs 9000 rad (in 5 fractions over 7-9 days) as the definitive treatment of choroid melanoma will be continued. Further, we expect to initiate a study of proton therapy of the para-aortic node region. With respect to technical ability our intent is to install the therapy modified EMI 7070 CBT unit and prepare programs for the use of the VAX computer (permitting almost immediate CBT scans in any plane in the section of the body under study). Radiobiological studies will be expanded to: 1) Evaluate efficacy of radio-protective compounds applied topically to the skin and 2) assess the relationship between treatment volume and tolerance.

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200. RADIATION THERAPY ONCOLOGY GROUP

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The Radiation Medicine Unit of the University of Southern California School of Medicine, through its facilities at the Los Angeles County USC Medical Center and affiliated institutions, intends to continue to expand its participation in individual institutional and joint clinical studies sponsored by the Radiation Therapy Oncology Group.

In the period of the previous project, Grant Number 1R01 CA 18259-03, USC entered 1221 patients onto RTOG studies, and for the most recent yearly reporting period available, calendar year 1977, ranked high in accession to RTOG studies, eight out of thirty-six, and first or second in three of the most active studies, Breast Stage I and II, Bone Metastases, and Brain Metastases. Work in the latter area resulted in a publication by Frank Hendrickson, M.D., 'The Optimum Schedule for Palliative Radiotherapy for Metastatic Brain Cancer.' Records of case accrual for the first 10 months of 1978 indicate an increased rate above 1977. USC faculty authored the active RTOG protocol, Irradiation of Adeno-Carcinoma of the Prostate, Stage B with Positive Pelvic Nodes, 75-06.

During this same period, the research team of the USC Radiation Medicine Unit activated studies evaluating the immune status of radiation therapy patients. In the period of the current application this experience will be expanded to study active immunomodulation in RTOG 78-11 Levamisole and Radiation Therapy in Lung Cancer. During the same period, USC will expand its studies of radiosensitizers, through continued accession to RTOG misonidazole studies, and of hyperthermia both externally and interstitially in combination with irradiation. A network of affiliating institutions has been formed to markedly increase patient accession and to enhance the impact of the RTOG program on the community cancer patient.

201. CHILDREN'S CANCER STUDY GROUP (CCSG)

Ablin, A. R., Wara, W., Matthay, K., Zoger, S., Kushner, J. H., Mentzer, W., deLorimier, A., Harrison, M., Rosenau, W., Univer-

sity of California, San Francisco Campus, School of Medicine, Dept. of Pediatrics, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

OBJECTIVES: 1. Our major objective during this grant period is to meaningfully contribute to the design, execution and evaluation of protocols of the Children's Cancer Study Group. Group studies, which are chaired by members of our oncology team, are, of course, of special interest to us and we plan to devote our major energies to these areas, i.e., germ cell tumors, hepatomas, interferon cell surface characteristics in ANLL, pneumococcal vaccine, half-body sequential radiation for relapsing histiocytosis, medulloblastoma, misonidazole with radiation therapy. 2. During this grant period, we plan to develop new strength in laboratory oncology, extending our interests beyond the clinic and into areas which are compatible with the Group's interests, specifically in the areas of cell surface characteristics of ANLL and closer examination of white cell function in the pediatric patient with malignancy and infection. 3. To participate in the evaluation of phase II agents in the treatment of childhood malignancies. 4. To make significant contributions to the Group effort in the pursuit of its goals in both the scientific and the administrative areas. 5. To further develop and improve the multidisciplinary approach to problem solving in childhood clinical cancer research both in our institution and in those in our referral area.

202. RADIATION THERAPY ONCOLOGY GROUP

Rubin, P., University of Rochester, School of Medicine & Dentistry, Dept. of Radiation Oncology, *601 Elmwood Ave., Rochester, New York*, 14627, United States of America

The RTOG is a cooperative group of radiation therapy research and clinical centers organized for the purpose of conducting joint clinical investigations. The Division of Radiation Oncology of the University of Rochester Cancer Center is one of the member institutions of the RTOG and the Office of Protocol Design and of the Vice-Chairman are located within it. A listing of the major RTOG objectives and progress that has been made by the group, both nationally and at this institution, is described and includes: case accession, new research areas, standardized treatment planning, quality control, neutron clinical investigation, study registries and forms, and the interaction of the RTOG with other cooperative groups. The protocol design activities of the RTOG are reviewed through two schemes: First, major radiation themes which can apply to many disease sites: time dose factors, radiosensitizers, systemic irradiation, high LET irradiation, optimal time of radiation therapy and chemotherapy, hyperthermia, combining innovations in radiation therapy and mathemtical modeling; second, disease sites in terms of unique specific studies, especially studies of brain, lung, head and neck, genitourinary and metastatic disease. The activities of the Division of Radiation Oncology in protocol accession and the studies which are chaired by members of this institution are reviewed.

203. RADIATION THERAPY ONCOLOGY GROUP

Goodman, R. L., Littman, P., Glick, J., Bloch, P. H., Carabell, S. C., University of Pennsylvania, School of Medicine, Dept. of Radiation Therapy, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

The University of Pennsylvania (UP) is a member of the Radiation Therapy Oncology Group (RTOG). It not only contributes large numbers of patients, especially those with head and neck tumors, but both radiation therapy and non-radiation therapy specialists contribute time and effort in committee work and in providing consultative services. Thus, the University of Pennsylvania exemplifies in its activities a commitment to the interdisciplinary approach to patients with oncological diseases and assists RTOG, a cooperative group of radiation therapy research and clinical centers, in the conduct of joint clinical investigations. The group conducts randomized clinical trials and other cooperative clinical studies to advance knowledge in the cure and control of cancer by gathering adequate numbers of patients necessary for the answer to important clinical questions. It also makes possible the collection of long-term data with regard to radiation complications and patient survival, and promotes multi-disciplinary care by integrating its study programs with those of other disciplines. In that way, it also enriches training programs in oncology as well as radiation therapy.

204. PI MESON RADIOTHERAPY OF BRAIN GLIOMA (HUMAN)

Kligerman, M. M., University of New Mexico, School of Medicine, Dept. of Radiology, *University Hill N.E., Albuquerque, New Mexico*, 87131, United States of America

This is part of a broader project. A summary of this subproject is not available.

205. MEASUREMENT OF NORMAL TISSUE CHANGE IN THERAPEUTICALLY IRRADIATED BRAIN (HUMAN)

Kramer, S., Thomas Jefferson University, Jefferson Medical College, Dept. of Radiation Therapy & Nuclear Medicine, *1025 Walnut St., Philadelphia, Pennsylvania*, 19107, United States of America

This is part of a broader project. A summary of this subproject is not available.

206. SURVIVAL, PROGNOSIS - RADIATION THERAPY FOR BRAIN TUMORS (CHILDREN)

Danoff, B., Thomas Jefferson University, Jefferson Medical College, Dept. of Radiation Therapy & Nuclear Medicine, *1025 Walnut St., Philadelphia, Pennsylvania*, 19107, United States of America

This is part of a broader project. A summary of this subproject is not available.

207. INTRATHECAL RADIOGOLD IN MENINGEAL TUMORS (CHILDREN)

Hahn, E. W., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

3. IMMUNOTHERAPY

208. AUTOREACTIVE CELLS IN TUMOR PATIENTS

Klein, E., Vanky, F., Masucci, G., Argov, S., Patarroyo, M., Karolinska Institutet, Dept. of Tumor Biology, *Solnavagen 1, S10401 Stockholm 60, Sweden*

Patients will be investigated with regard to their immunological reactivity against their own tumors (lung tumors, osteosarcoma, astrocytomas) using two in vitro assays: ATS-autologous tumor stimulation which measures blastogenesis of lymphocytes exposed to the biopsy cells and ALC-autologous lymphocyte cytotoxicity exerted towards biopsy cells. In our past research a certain proportion of patients have exhibited such reactivities. In a further step the optimal conditions for generating cytotoxic cells in vitro by cocultivation with biopsy cells will be explored. It is possible that such in vitro generated reactive cells can be exploited in the future for immunotherapy.

Tumor patients with the same and different histopathology will be tested for cross-reactivity in the ATS and ALC assays. Preliminary experiments indicated that when biopsy preparations highly enriched for tumor cells are used, reactivity due to alloantigens does not occur.

A prerequisite for the above delineated studies is the separation of biopsy into tumor and reactive cell components. The methodology has been worked out in our laboratory. This also allows the study of the nature of infiltration and the function of infiltrating cells in vitro. Thus information about in situ tumor immunity will also be obtained.

209. PRELIMINARY CLINICAL STUDIES ON INTRATHECAL AUTOLOGOUS LYMPHOCYTE INFUSIONS IN MALIGNANT GLIOMA PATIENTS

Neuwelt, E. A., Clark, W. K., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Neurosurgery Section, *4500 S. Lancaster Rd., Dallas, Texas*, 75216, United States of America

The best current treatment of human malignant gliomas consists of surgery, irradiation and chemotherapy. Median survival is ten months from time of diagnosis. The lack of a cellular immune response in the brain and the relative inability of lymphocytes to penetrate the blood brain barrier (BBB) of glioma patients could in part, account for the poor prognosis. Tissue culture studies indicate clearly that autologous lympho-

cytes are capable of destroying malignant glioma cells, and clinical studies suggest that (1) a direct correlation exists between lymphocytic infiltration and prolongation of survival and (2) that even a single infusion of autologous lymphocytes into the tumor bed may enhance survival. We are examining the effects of treatment by repetitive intrathecal infusion of autologous lymphocytes in patients with malignant gliomas. Cells obtained from the patient via a cell separator and purified are injected into the subarachnoid or ventricular space at two to four week intervals. If adequate communication between the tumor bed and the subarachnoid space is not present (as determined by metrizamide cisternography), the lymphoid cells will be infused directly into the tumor bed. Hazards from repetitive lumbar puncture or from break in sterile techniques have been small in 78 infusions. Eleven patients have been placed on various similar but not standardized protocols with no evidence of toxicity noted thus far, and objective evidence of therapeutic efficacy in at least one patient has been observed. If the above treatment proves successful, not only will the treatment of humans with malignant gliomas be improved, but the techniques may also prove applicable to a variety of neurologic diseases, i.e., viral and fungal meningitis. Now that a standard treatment regimen has been developed, our plan is to conduct a Phase II (therapeutic efficacy) trial with 15-20 patients, and compare the results with standard therapy (surgery and radiotherapy) as published by the Brain Tumor Study Group.

210. TRANSFER FACTOR IMMUNOTHERAPY IN CANCER - CLINICAL APPLICATIONS AND BASIC PROPERTIES

Vetto, R. M., Burger, D. R., Vandenbark, A. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Surgical Service, *Sam Jackson Park Rd., Portland, Oregon*, 97207, United States of America

Progress on this project has been achieved in 2 major areas: (1) Fractionation and characterization of transfer factor (TF). (2) Detection of reactivity to tumor antigens in cancer patients by the leukocyte adherence inhibition (LAI) test, and by a new approach, the antigen binding cell assay.

Transfer factor activity measured by the transference of delayed type skin test reactivity from immunized donors (humans or calves) has been characterized by chemical separation (high pressure liquid chromatography and isoelectric focusing) and enzymatic treatment (alkaline phosphatase, pronase, proteinase K, and phosphodiesterase I and II). Results suggest TF separates as a dislinite entity of low molecular weight and strongly negative charge and has both peptide(s) and nucleotides as structural components. Melanoma neuroblastomic and leukocyte adherence inhibition test used to evaluate immunifying patients with squamous cell carcinoma of the head and neck and families with a high incidence of melanoma has detected higher levels of reactivity within specific tumor groups to their appropriate tumor extract. The mechanism involved in this assay includes reactions by multiple cell types, and serum factors from immune donors which can arm nonimmune cells to be reactive to specific tumor extracts. The antigen binding cell assay - which measures formation of rosettes between immune cells and tumor antigen-conjugated red cells - has detected an increased percentage of mononuclear cells which can bind squamous carcinoma antigen in patients with advanced primary tumor but not in patients with recurrence or in controls.

211. BRAIN TUMOR IMMUNOTHERAPY (HUMAN)

Mahaley, M. S., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Surgery, *Manning Dr., Chapel Hill, North Carolina*, 27514, United States of America

This is part of a broader project. A summary of this subproject is not available.

212. ACTIVE IMMUNOTHERAPY FOR PATIENTS WITH ANAPLASTIC GLIOMAS

Mahaley, M. S., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Surgery, *Manning Dr., Chapel Hill, North Carolina*, 27514, United States of America

This is part of a broader project. A summary of this subproject is not available.

213. INTRATUMOR LYMPHOCYTE INFUSION FOR RECURRENT BRAIN TUMORS

Rekate, H. L., University Hospitals of Cleveland, *2065 Adelbert Rd., Cleveland, Ohio*, 44106, United States of America

This is part of a broader project. A summary of this subproject is not available.

B. MULTIPLE MODALITY THERAPY

1. CHEMOTHERAPY AND RADIOTHERAPY

214. RADIOTHERAPY FOR CENTRAL NERVOUS SYSTEM TUMORS

Ghilezan, N. I., Milea, N. I., Tamburlini, V. S., Mogut, D. I., Oncological Inst. of Cluj-Napoca, Cobalt, *Republicii Nr. 34-36, R3400 Cluj-Napoca, Romania*

OBJECTIVE: To determine the indications for postoperative radiotherapy plus chemotherapy according to site and histology for central nervous system tumors.

APPROACH: Patients with histologically confirmed diagnosis of central nervous system tumors are eligible for study. The local failures are analyzed according to histology, tumor volume, the quality of surgery and the irradiation technique. The postoperative irradiation will be supplemented with chemotherapy, i.e., BCNU.

215. COMBINED CCNU AND RADIOTHERAPY IN MALIGNANT BRAIN TUMORS

Ojala, A. T., Mantyla, M., Hakala, T., Wallinheimo, H., Tampere Central Hospital, Dept. of Radiotherapy, *36280 Pikonlinna, 33520 Tampere 52, Finland*

OBJECTIVE: To study: 1) The results of whole brain irradiation versus local tumor area irradiation in malignant brain tumors and (2) the results of combined CCNU and radiotherapy versus radiotherapy alone in malignant brain tumors.

APPROACH: Participating institutions are Tampere and Jyvaskyla Central Hospitals. Adult patients with malignant brain tumor will be randomized into four different treatment groups: 1) Whole brain radiotherapy with CCNU; 2) whole brain radiotherapy without CCNU; 3) local tumor area radiotherapy with CCNU; 4) local tumor area radiotherapy without CCNU. The results will be evaluated in terms of recurrent free period and survival time.

216. CLINICAL EVALUATION OF AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PEDIATRIC ONCOLOGY

Wagner, H. P., Baumgartner, C., Bucher, U., Imbach, P., Universitat Bern, University Hospitals, Dept. of Pediatrics, *c/o Tiefenauspital, 3004 Bern, Switzerland*

High-dose VAC-type chemotherapy (CT) followed by total body irradiation (TBI), 600 rads, and reimplantation of cryopreserved, autologous bone marrow (0.5 x 10 to the fifth power CFU-c per kg body weight, or more) will be used for the treatment of children with relapsing, advanced Ewing's sarcoma, neuroblastoma, non-Hodgkin's lymphoma, yolk sac tumor or leukemia.

Based on the results obtained, an attempt will be made to define indications for an early use of the aforementioned procedures in the treatment of children with cancer known to have a poor prognosis. As of November 1980, 13 children have received cryopreserved autologous bone marrow after CT and TBI. Time of observation has been too short for evaluation.

217. CONTROLLED CLINICAL TRIALS FOR BREAST AND GASTROINTESTINAL CANCER AND BRAIN TUMORS

Veronesi, U., Istituto Nazionale per lo Studio e la Cura dei Tumori, *1 Via G Venezian, 20133 Milan, Italy*

Controlled clinical trials for breast and gastrointestinal cancer and brain tumors using the following protocols: Controlled study of BCNU, CCNU, and Irradiation in the Treatment of Malignant Glioma; Controlled Study with Sequential Multiple Drug Combinations for Advanced Breast Cancer; Combination Chemotherapy with CMF as an Adjuvant to Radical Mastectomy: 12 vs. 6 cycles; Controlled Study with Combined Chemotherapy-Radiotherapy vs. Chemotherapy-Surgery in T3b-T4 Breast Cancer; Surgically Operable Rectal Carcinoma, Stages B2 and C; and Adjuvant Therapy for Adenocarcinoma of the Colon following Clinical Curative Resection.

GOAL OF AGENCY: Research activities aimed at specific disease or problem areas.

218. CLINICAL STUDIES ON SENSITIZERS OF HYPOXIC TUMOR CELLS

Urtasun, R. C., Turner, A. R., Rabin, H., Miller, J. D., University of Alberta, Dr. W.W. Cross Cancer Inst., *11560 University Ave., Edmonton, Alberta*, T6G 1Z2, Canada

OBJECTIVE: To decrease the toxicity of these drugs by using methods that could alter the pharmacokinetics or metabolism, i.e., use of Dexamethasone (Decadron). To uncover potential toxicities that could occur when combining these drugs with other modalities. To predict the best timing of radiation treatment in different tumors. To assess whether there is improvement in patient survival when comparing this new approach with conventional treatment methods in patients harboring tumors of extremely poor prognosis. To improve the knowledge of the bioavailability of the drug and its pharmacokinetics when administered by different routes. To select tumors most likely to be benefited by these approaches.

APPROACH: The effectiveness of using radiosensitizers plus unconventional fractionated radiation will be compared to the best available conventional radiation using patients with glioblastomas. The comparative effectiveness of metronidazole versus misonidazole will also be assessed in this pilot clinical trial.

A modification of the neurotoxicity of these drugs will be assessed by the use of Decadron (Dexamethasone). The pharmacokinetics of misonidazole in solid tumor tissues will be assessed by performing multiple biopsies in 15 patients with different tumors. The drug will be administered p.o. and intravenously to compare bioavailability. Bone marrow toxicity to metronidazole and misonidazole will be assessed in humans by the colony forming units assay (CFU-C).

219. SUPPORT OF CLINICAL TRIALS IN THE DIVISION OF ONCOLOGY AT SUNNYBROOK MEDICAL CENTRE

Osoba, D., Sunnybrook Hospital Medical Centre, Division of Oncology, *2075 Bayview Ave., Toronto, Ontario*, M4N 3M5, Canada

Patients with histologically proven cancer are being entered into a variety of clinical trials. These include: 1. Chemotherapy of small carcinoma of the lung with cyclophosphamide, adriamycin and vincristine, including prophylactic brain irradiation. 2. Chemotherapy of disseminated non-small cell carcinoma of the lung with cyclophosphamide, adriamycin and cis-platinum or 5-fluorouracil, adriamycin and mitomycin-C. 3. Immunotherapy of resectable stage I or II non-small cell carcinoma with Freund's adjuvant and tumor cell wall antigen. 4. Immunotherapy of stage I or III resected malignant melanoma with either BCG or levamisole or BCG and levamisole. 5. Chemotherapy of advanced renal adenocarcinoma with vincristine and hydroxyurea. 6. Chemotherapy of stage III or IV epithelial carcinoma of the ovary with either melphalan or hexamethylmelamine, cyclophosphamide, adriamycin and 5-fluorouracil or cyclophosphamide, adriamycin and cis-platinum. 7. A phase I trial of the capacity of oral folinic acid to prevent toxicity of oral methotrexate. 8. Combined trial of chemotherapy with CCNU and hydroxyurea and irradiation in grade III or IV astrocytoma. Approximately 50-70 patients/year are entered in the above trials.

220. MALIGNANT GLIOMA RADIOTHERAPY PLUS BCNU CHEMOTHERAPY COMPARED TO RADIOSENSITIZED RADIOTHERAPY PLUS BCNU CHEMOTHERAPY

Cox, J. D., Byhardt, R. W., Kun, L. E., Greenberg, M., Komaki, R., Gunn, W., Rangala, N., Wilson, J. F., Holoye, P. Y., Larson, S. J., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *5000 W. National Ave., Wood, Wisconsin*, 53193, United States of America

PROJECT OBJECTVES: To establish the relapse-free mean survival time and overall survival in patients treated with misonidazole radiosensitized irradiation combined with BCNU chemotherapy compared with standard irradiation combined with BCNU chemotherapy.

PLAN: To determine the incidence of any increased toxicities or brain complications when misonidazole radiosensitized irradiation is combined with BCNU chemotherapy.

METHOD: Patients will receive BCNU 80 mg/M2 I.V. on days 3, 4, and 5 of the first week of radiotherapy, then BCNU 80 mg/M2 I.V. x 3 days q. 8 weeks beginning day 64. Radiotherapy will be given as follows: 200 rad/day x 5 days a week x 6 weeks (6000 rad to the whole brain). Radiosensitized radiotherapy plus chemotherapy: Patients will receive oral misonidazole (2.5 grams per M2) four hours prior to a 400 rad fraction of whole brain irradiation each Monday for 6 consecutive weeks. BCNU 80 mg/M2 I.V. will be given on days 3, 4, and 5 of the first week of radiotherapy, then BCNU 80mg/M2 I.V. x 3 days q. 8 weeks beginning day 64. During that time irradiation without radiosensitization will continue with 150 rad each Tuesday, Thursday and Friday of these 6 consecutive weeks. Thereafter the brain will be treated with an additional 180 rad in 5 fractions over one week with radiosensitization.

RESULTS REACHED TO DATE: None. One patient has been treated with radiosensitized radiotherapy plus BCNU chemotherapy with no adverse reactions or complications.

PUBLICATIONS: None. Protocol is still currently active.

221. RANDOMIZED PHASE III PROTOCOL FOR EVALUATION OF MISONIDAZOL COMBINED WITH RADIATION IN TREATMENT OF PATIENTS WITH BRAIN METASTASIS

Abdel Dayem, H. M., Spaulding, M., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *3495 Bailey Ave., Buffalo, New York*, 14215, United States of America

The work on this project has actually started and one patient has been included as per our previous memorandum. The initial report for this protocol is the subject of this memorandum.

This protocol compares two fractionations of radiation therapy to patients presenting with brain metastasis from different primaries, excluding lymphomas. It compares two regimens of radiation: (1). Three thousand rads to the brain in two weeks at daily increments of 300 rads for 10 increments. (2). Fractionation of 500 rads twice weekly for 6 increments (total of 3,000 rads in 3 weeks in 6 increments). These two fractionations are compared with and without Misonidazol, a drug which has been found to be a radiosensitizer.

Patients are randomized by the Central Office of Radiation Therapy-Oncology Group, and are assigned to one of the four arms of the treatment. So far, only one patient has been entered into this study.

222. RADIATION THERAPY ONCOLOGY GROUP

Chang, C. H., Tretter, P. K., Harisiadis, L., Sung, D. I., Oster, M., Housepian, E. M., Abramson, M., Rankow, R., Wolff, J., Perzin, K., Kessaris, N., Columbia University, College of Physicians & Surgeons, Dept. of Radiology, *630 W. 168th St., New York, New York*, 10032, United States of America

This research proposal is to continue participation on a cooperative group of Radiation Therapy Centers in the United States (known as Radiation Therapy Oncology Group) for the purpose of conducting joint clinical investigation in an endeavor to advance knowledge in the curative and palliative management of cancer.

This institution has participated in several protocols in clinical trial: Phase I/II protocol for evaluation of Misonidazole Combined with Radiation in the Treatment of Patients with Malignant Glioma. Phase I/II Study of Hyperthermia Treatment Technique and Tolerance of Skin and Subcutaneous Tissues to a Temperature of 40-45 degrees C, and other protocols which were reported previously.

Several Phase III protocols evaluating hypoxic cell radiation sensitizer have just been activated and we are participating in these studies. These include: Phase III Protocol for Malignant Glioma -Standard Radiotherapy Plus BCNU Chemotherapy Compared to Radiosensitized Radiotherapy Plus BCNU Chemotherapy; Phase III Study to Compare Misonidazole Combined with Irradiation or Radiation Therapy Alone in the Treatment of Locally Advanced (Stage III) Non-Oat Cell Lung Cancer.

223. SOUTHWEST ONCOLOGY GROUP PROTOCOL 7811, BRAIN METASTASES PROTOCOL, PHASE III

Coltman, C. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *San Antonio, Texas*, 78284, United States of America

This protocol is designed to determine the effectiveness of combined radiotherapy and metronidazole (Flagyl) in the treatment of patients with brain metastases from primary malignancies outside the central nervous system compared with radiation therapy alone. Flagyl is a known radiation sensitizer. The end point will be objective response (brain and/or cat scan improvement) and/or increase in functional neurological level and duration of response. The study will also be designed to test the toxicity of multiple dose administration of metronidazole and radiation therapy. Patient must have histological proof of primary malignancy. There must be clinical suspicion of brain metastases documented by isotope brain scan and/or cat scan and/or neurologic status level of 2 to 4. Patients will not receive concomitant chemotherapy with agent known to pass the blood brain barrier per metronidazole in combination with the whole brain. Radiation has been used in patients with primary brain tumors and shown to have an enhancement of survival. This study is designed to determine that effect in patients with brain tumors metastatic from sites outside the central nervous system.

No patients have been entered on this study to date.

224. METRONIDAZOLE AND LARGE FRACTION BRAIN IRRADIATION FOR GLIOBLASTOMA

Kapp, D., Wagner, F., Chen, M., Son, Y., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *W. Spring St., West Haven, Connecticut*, 06516, United States of America

Patients with glioblastoma multiforme are currently treated with resection followed by conventional radiotherapy with an extremely low incidence of tumor control. There is radiobiological evidence that larger individual fractions of irradiation (e.g., 600 rads) might be more effective in killing glioblastoma cells. Since the electron affinic compound metronidazole has been shown to have in vitro, in vivo and clinical activity in sensitizing hypoxic tumor cells to irradiation, it would be reasonable to study the use of this drug in combination with large individual fractions of irradiation for the treatment of patients with glioblastoma multiforme. A pilot study is indicated to better define the toxicity to normal central nervous system tissues of such combined therapy and to determine the incidence of local control of this tumor and survival using this treatment.

225. CRANIAL VAULT RADIOTHERAPY AND COMBINATION INTRATHECAL CHEMOTHERAPY IN THE TREATMENT OF MENINGEAL CARCINOMATOSIS

Bunn, P. A., Minna, J. D., Cohen, M. H., Ihde, D. C., Matthews, M. J., Myers, C., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *50 Irving St. N.W., Washington, District of Columbia*, 20422, United States of America

The specific aim of this study is to determine whether addition of the alkylating agent, thio-TEPA, to the standard regimen of craniospinal radiotherapy, IT-MTX, and dexamethasone results in improved survival of patients with meningeal carcinomatosis. Diffuse involvement of the leptomeninges with tumor occurs commonly in patients with metastatic breast carcinoma, metastatic lung cancer, melanoma and histiocytic lymphoma. Co-existing CNS mass lesions are seen in 25%-75% of patients with meningeal carcinomatosis. The prognosis of patients with meningeal carcinomatosis is poor. In recent series, the median survival for patients with a variety of primary tumors was less than 6 weeks from the time of diagnosis of the meningeal involvement. Currently used treatment modalities involve the use of cranial (or craniospinal) radiotherapy, intrathecal methotrexate, or combinations of the two. Sixteen patients with breast cancer and meningeal carcinomatosis have recently been treated at the Memorial Hospital. One out of four responded to IT-MTX alone; three out of eight responded to craniospinal radiation alone; and three out of four responded to a combination of craniospinal radiotherapy and intrathecal methotrexate. Systemic chemotherapy, even with drugs that cross the blood-brain barrier, does not appear to be effective.

226. GENERAL CLINICAL RESEARCH CENTER

Bondurant, S., Utiger, R. D., Clemmons, D. R., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Medicine, *Manning Dr., Chapel Hill, North Carolina*, 27514, United States of America

This unit serves as a facility for carrying out clinical research projects on hospitalized patients and normal subjects. Examples of projects in this unit include studies of Hypercoagulability and Thrombosis; Growth Hormone in Pituitary Disorders; Genetic Disorders of Central Nervous System; Alcoholic Hepatitis; Bleeding Esophageal Varices; Physiology and Kinetics of Leukocytes; Intestinal Peptide Hydrolases; Chronic Active Hepatitis; Epidermolysis Bullosa; Home Intravenous Hyperalimentation; BCNU, Medrol, Procarbazine, and Radiotherapy in Treatment of Brain Tumors; Gross Peptic Esophagitis; Muco-Cutaneous Candidiasis; Bartters Syndrome; Dietary Protein, Calcium and Phosphorus; Propylthiouracil and Methimazole in Early Treatment of Hyperthyroidism; Caloric Deprivation and Weight Reduction in Diabetic Control; Overnutrition and Thyroid Hormone Regulation; Adenocarcinoma -Unknown Primary Evaluation; Pathogenesis of Bullosa Pemphigoid; Ectopic Hormones in Non-Endocrine Malignancy; Thyrotropin Physiology; Effects of Smoking on Codeine Disposition; Hyperbaric Oxygenation Treatments for Extracranial-Intracranial Arterial Anastomosis; Immunotherapy for Patients with Anaplastic Gliomas; Adenine Therapy for Fanconi's Anemia; Clinical-Pathologic Correlation of Ocular Sarcoidosis; Acyclovir Administered to Immunocompromised Children with Varicella Zoster, Herpes Simplex and Cytomegalovirus Infections; Somatomedin-C in Acromegaly; Lymphokine Treatment of Laryngeal Papillomatosis.

227. CLINICAL RADIATION RESEARCH CENTER

Rubin, P., University of Rochester, School of Medicine & Dentistry, Depts. of Radiology/Medicine/Surgery, *601 Elmwood Ave., Rochester, New York*, 14627, United States of America

The Clinical Radiation Research Center (CRRC) programs constitute the major investigative activities of the Division of Radiation Oncology of the University of Rochester Cancer Center (URCC). The unique aspect of the CRRC and the URCC is the integration of research in the clinical sphere and the laboratory setting. The Radiation Oncology Clinical Investigative Program of the CRRC utilizes the laboratory to model and simulate clinical problems to increase understanding of the pathophysiologic effects of irradiation in humans with malignant disease. The Radiobiology and Biophysics Program investigates methods for combining radiation and other modalities for effective cell killing by the use of tumor models in vitro and in vivo. When promising results are found in the laboratory, pilot protocols are written and implemented in the University of Rochester Medical Center through the collaboration of the divisions of Radiation and Medical Oncology. The Radiation Physics Program provides special support for the other programs and conducts its own research in treatment planning, developing clinical guidelines, mathematical optimization, and applying CT scanning imagery. Personnel and programs interact at different levels and combine efforts in various theme projects such as combined modality treatment of brain tumors, radiosensitizers, and bone marrow regeneration.

228. POSITRON EMISSION TOMOGRAPHY OF THE CNS

Posner, J. B., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

The overall goal of this program project is the elucidation of the effects of human neurologic disease on brain metabolism. A primary focus of the CORE Proposal is malignant disease, specifically the physiologic and metabolic consequences of hemispheral neoplasms and peritumoral edema. Other major elements of the CORE proposal include (1) the effects of radiation and chemotherapeutic agents on normal brain tissue; (2) the delineation of the intraparenchymal regions of abnormal capillary pemeability; and (3) the determination of regional equilibrium tissue concentrations of phenytoin in noral volunteers and seizure patients. An equaaly important focus of this application is the study of cerebral ammonia metabolism, and the study of cerebral metabolism and physiology in the aged and demented. A proposed collaboration with Rockland Research Institute concerns the relative importance of specific pathways of carbo-

hydrate intermediary metabolism in normal volunteers and psychiatric inpatients. The development of new radiopharmaceuticals and original biochemical and mathematical models will proceed pari passu with the application of existing quantitative methods (for the determination of rCBV, rCBF, rCMRO2 and rCMRGlu) to the above mentioned neurological subpopulations.

229. CHEMOTHERAPY OF MALIGNANCY IN PARTICIPATION WITH ECOG

Zucker, S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *Middleville Rd., Northport, New York*, 11768, United States of America

During the past year, we have treated 34 cancer patients according to protocols developed by the Eastern Cooperative Oncology Group (ECOG). These protocols include chemotherapy and/or radiotherapy. Both standard and experimental drugs are employed. The types of cancer protocols employed include: lung, gastrointestinal, liver, prostate, and brain cancers; leukemias, lymphomas, and myelomas. On-study, interim and final forms have been sent to ECOG. Our response rates and complications have been similar to those reported by the entire group with no extraordinary problems noted. This program will be continued during the next year with an attempt to increase the patient accrual rate.

230. RADIATION THERAPY ONCOLOGY GROUP

Fu, K. K., Phillips, T. L., Luk, K., Hill, D., University of California, San Francisco Campus, School of Medicine, Dept. of Radiation Oncology, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

Support requested in this application is for the ongoing and expanding participation of the Department of Radiation Oncology of the University of California in San Francisco in the activities of the Radiation Therapy Oncology Group. Comprised of three hospitals, Moffitt, Mt. Zion and Franklin, the Department is staffed by eleven full-time faculty members, treating 2000 new patients per year. The Department has participated in the Group since 1972, entering approximately 150 patients into the various Group protocols, pilot studies and registry protocols during the last year. The Principal Investigator is a member of the Head and Neck Committee, Combination Radiotherapy and Chemotherapy Sub-Committee and Hematopoietic Sub-Committee of the Radiation Therapy Oncology Group. The Associate Principal Investigator is Chairman or the Radiobiology Committee and a member of the Publication Committee, Experimental Design Committee, Lung Sub-Committee, Metastases Sub-Committee, Radiation Injury Sub-Committee, Hyperthermia Sub-Committee and Chairman of the Radiosensitizer/Protector Committee and all staff members serve on the working parties. The group has produced protocols for head and neck cancer, non-Hodgkin's lymphoma, brain metastasis, and hypoxic cell sensitizers, as well as providing input into other protocols. Support through this application will allow RTOG participation by the entire Department and its affiliated institution, the University of California, San Diego, Division of Radiation Oncology and increase the case accession rate to 175-200 new patients per year into the various RTOG protocols. Extensive continuing research into chemical and thermic modification of radiation effects and high LET radiotherapy is expected to lead to innovative protocols and correspondingly to increased accession of patients into studies sponsored by the RTOG.

231. COMBINED CHEMO- AND RADIOTHERAPY OF BRAIN TUMORS

Bagshaw, M. A., Stanford University, School of Medicine, Dept. of Radiology, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

This is part of a broader project. A summary of this subproject is not available.

232. EFFECTS OF RADIATION AND CHEMOTHERAPY ON BRAIN TUMORS (HUMAN, ANIMALS)

Sung, J. H., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Therapeutic Radiology, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

This is part of a broader project. A summary of this subproject is not available.

233. BCNU, MEDROL, PROCARBAZINE AND RADIOTHERAPY FOR BRAIN GLIOMAS (HUMAN)

Mahaley, M. S., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Surgery, *Manning Dr., Chapel Hill, North Carolina*, 27514, United States of America

This is part of a broader project. A summary of this subproject is not available.

234. COMBINED THERAPY OF GLIOBLASTOMA

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This is part of a broader project. A summary of this subproject is not available.

235. BRAIN TUMOR AND TREATMENT WITH MULTIMODAL THERAPY

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This is part of a broader project. A summary of this subproject is not available.

2. CHEMOTHERAPY AND IMMUNOTHERAPY

236. IMMUNO-CHEMOTHERAPY OF MALIGNANT BRAIN TUMORS

Paoletti, P., Knerich, R., Butti, G., Locatelli, D., Robustelli della Cuna, G., Universita degli Studi di Pavia, Clinica Neurochirurgica, *65 Corso Strada Nuova, 27100 Pavia, Italy*

OBJECTIVES: Objectives of the study are: a) To determine in a controlled, prospective and randomized study if the combination of radiotherapy (RT), chemotherapy (BCNU and CCNU) and immunotherapy with BCG is superior to treatment with radiotherapy and chemotherapy (BCNU or CCNU) alone in terms of response rate, prolongation of survival time and improvement of quality of life. b) To determine relationships between immunologic parameters, performance status, response rate and survival.

APPROACH: Patients are stratified into two groups: Category A: patients with operable malignant brain tumors; Category B: patients with inoperable malignant brain tumors.

Patients of Category A are randomized to RT plus BCNU (control arm) or RT, BCNU and BCG; patients of Category B to RT plus CCNU (control arm) or RT, CCNU and BCG.

PROGRESS: Since July 1, 1979, (beginning of the study) we have studied 8 patients ranging from 31 to 68 years of age, 6 males and 2 females. Five patients underwent surgery for removal of the tumoral mass (Category A). Three were randomized to the control group and two to adjuvant immunotherapy. Three patients were diagnosed as inoperable (Category B) and thus randomized: 2 to the control arm, 1 to the adjuvant immunotherapy. During treatment 4 patients died, 2 of intervening causes and 2 of tumor regrowth.

Due to the limited number of patients entered into the study to date it is presently impossible to draw conclusions relative to the research objectives.

237. HUMAN SPECIFIC IGG ATTACHED TO RUBIDOMYCIN AS A NEW APPROACH TO TREATMENT OF METASTIC NEUROBLASTOMA

Castello, M. A., Hobbs, J., La Greca, G., Mancuso, G., Clerico, A., Melino, G., Universita degli Studi di Roma, Dept. of Pediatrics, *Rome, Italy*

OBJECTIVE: Considering that stage IV of neuroblastoma has normally a fatal course with standard treatment, it is proposed to attempt a new type of therapeutical approach. It consists in the use of human specific IgG attached to rubidomycin that has shown in vitro a 100% cytocidal effect on the neuroblastoma cells.

APPROACH: All the children with neuroblastoma, stages III and IV, sent to the Pediatric Oncology Center of the Pediatric Department of Rome University are included in the present research.

Neuroblastoma cells are being cultured directly from the tumor mass or from the bone marrow of the patient. They are then irradiated with 10,000 r and administered twice to one of the parents, chosen by his/her immunological response (MLR) to the tumor cells. After two weeks, specific IgG are obtained

from the plasma of the parent and attached to rubidomycin. The substance is then administered to the child. The effect of the therapy is assayed before and after the treatment.

238. INTENSIVE CHEMOTHERAPY AND IMMUNOTHERAPY IN NEUROBLASTOMA STAGES III AND IV - PHASE II STUDY

Waters, K. D., Ekert, H., Royal Childrens Hospital Research Foundation, Dept. of Hematology, *Flemington Rd., Melbourne, Victoria 3052, Australia*

HISTOLOGIC TYPE AND CLINICAL STAGE: Neuroblastoma - All paediatric patients with stage III and stage IV neuroblastoma are eligible for study.

TREATMENT MODALITIES: Vincristine, Cyclophosphamide, Adriamycin and Imidazole Carboxamide (DTIC). Transfer factor prepared from one of the parents if tumor immunity can be demonstrated in the parents using a migration inhibitor factor (MIF) assay.

PROTOCOL OUTLINE: The trial will open to all patients with stage III and stage IV neuroblastoma, who have not received any previous chemotherapy. The chemotherapy will be given as follows - Vincristine 1.5mg/m2 IV on days 1 and 5, Cyclophosphamide 600mg/m2 IV on day 1, Adriamycin 50mg/m2 IV on day 3, DTIC 200mg/m2 IV on days 1 to 5. This regimen will be repeated every 29 days. When remission is achieved transfer factor will be given on day 22 of each cycle. Observations during study will be - symptoms and physical findings, documenting size of main lesion; urine catecholamines every eight weeks until normal, than every twelve weeks; skeletal survey every eight weeks till clear, then every twelve weeks; bone marrow aspiration every 8 weeks (if positive initially) until negative, then as indicated clinically. MIF assays to assess anti-tumor immunity will be performed every 12 weeks in the patients treated with transfer factor to document any alterations in tumor immunity. Patients with progressive disease will be removed from study. Patients showing a partial response or static disease will remain on study until progressive disease occurs. Patients who demonstrate a complete response will remain on therapy for 24 months.

PROGRESS: Twenty patients entered on study, 13 patients died, 7 alive. Median survival time - 22 months. Study will continue, and it is planned to enter 4 or 5 patients per year.

239. USE OF THE CELL SEPARATOR IN THE TREATMENT OF SELECTED CANCER PATIENTS

Hobbs, J. R., Forrest, D., Samman, P. D., Retsas, S., Melino, G., University of London, Westminster Medical School, Dept. of Chemical Pathology, *17 Horseferry Rd., London SW1P 2AR, England, United Kingdom*

APPROACH: Human neuroblastomas, melanomas, or hypernephromas are cultured and farmed for up to six generations. Tumor cells irradiated with 10,000 rads are injected into informed patient. Samples are later tested against the relative tissue culture, and also against the normal leukocytes of the donor of the tumor. This use of one haplotype-identical volunteer results in few reactions against the normal leukocytes of the patient. The reaction against the tumor cells is assayed to see whether it is mainly humoral or cellular: if cellular, the patient may be treated with irradiated lymphocytes harvested from the volunteer, or with transfer factor prepared from such lymphocytes, or indeed with extracts of RNA similarly prepared. If humoral, we harvest large quantities of plasma from the volunteer, process it over cold alcohol (COHN) to isolate the gammaglobulin fraction, to which either chlorambucil or rubidomycin is conjugated. Checks are made for sterility and pyrogenicity before use.

PROGRESS: Over 120 human volunteers have been immunized with no side effects so far, and in almost every case a usable immune reaction against the tumor. Therapy with immune cells has only been attempted in eight patients, all of whom were 'unblocked' prior to receiving the cells, and in all cases an objective regression of tumor was observed, but was nearly always temporary. Attempts to educate the leukocytes of the patient with extracts from the volunteer have so far failed (? any recipient clones).

Chemoimmunotherapy with 'tumor-specific antibodies' coupled to cytotoxic drugs has now been used in 10 Stage I patients, achieving impressive objective regression of tumor, but thus far transient up to 15 months.

240. CANCER CHEMOTHERAPY AND IMMUNOTHERAPY PROTOCOLS, SWOG 7635, 7703, 7704

Coltman, C. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *San Antonio, Texas, 78284*, United States of America

SWOG 7703 - The purpose of this study is to compare the effectiveness of radiation therapy plus BCNU, radiation therapy plus DTIC, and radiation therapy plus procarbazine for remission induction, duration of remission, and survival in patients with malignant gliomas of the brain. A total of 6 patients from this institution were entered into this study. The study was activated in March of 1977 and remains open. The response rate for procarbazine is 18 percent, BCNU 33 percent, DTIC 30 percent, and there is no difference in survival.

SWOG 7704 - To compare the effectiveness of three intermittent plus chemotherapy combinations VMCP plus VCAP vs. VMCP plus VBAP vs. MP for induction of remission in previously untreated patients with multiple myeloma. Results will also be compared with other combination chemotherapy treatments in previous SWOG studies, especially VMCP treatment in SWOG 7418 and previous studies of MP combinations. For patients proven to have at least a 75 percent tumor regression after induction, to compare the value of 12 months of chemoimmunotherapy maintenance VMCP plus levamisole in comparison to VMCP alone. To establish baseline and serial data on immunologic status in these patient groups. This study began in March of 1977 and closed in August of 1979. A total of 8 patients from this institution were entered into the study. The response rates for the three regimens are 47 percent for VMCP plus VCAP, 41 percent for VMCP plus VBAP, and 38 percent for MP, no significant difference. The number of patients relapsed was smallest in the VMCP plus VBAP group. There is a difference in survival between the VMCP plus VBAP group vs.the MP group at the 5 percent level. This favors VMCP plus VBAP.

241. CHILDREN'S CANCER STUDY GROUP - A

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CCG has the expertise to combine whole group protocol studies with small intra-group investigations of tumor biology. Large scale clinical programs are needed to consolidate data accrual of the past decade. Included in small group studies are those utilizing data on experiments in nature, i.e., adenosine deaminase (ADA) deficiency; anti-cancer effects of interferon; anti-tumor antibodies (common ALL, neuroblastoma); transplantation (allogeneic, autologous) biology; autologous lymphocyte therapy; radiosensitizer therapy; limb salvaging techniques/aggressive metastatic surgical attack; and diagnostic imaging procedures. Induced ADA deficiency can be applied to the study of T-cell leukemia and to the nature of lymphoblast modulation. Interferon data can be applied to the study of non-T ALL, non-Hodgkin's lymphoma and osteosarcoma and to the nature of tumor cell death. Anti-(common) ALL antibody studies can be applied to the development of common ALL and non-T, non-B lymphoma protocols. Specific anti-neuroblastoma antibody data can be applied to the development of randomized studies comparing chemotherapy and chemotherapy plus adjuvant antibody therapy. Allogenic transplantation for AML can be expanded to relapsing high risk ALL and high risk lymphoma. Autologous marrow transplantation rescue is applicable for megadose therapy studies of leukemia, lymphoma and a variety of solid tumors and as a means of evaluating techniques for identifying occult tumor burdens. Similarly, autologous lymphocyte infusion into brain tumors can test the hypothesis that such malignancies occupy immunologically priviledged sites and in turn be incorporated into a randomized radiosensitizer therapy study. Conflicting osteosarcoma data can be resolved by comparing pre-op megadose therapy to surgery alone (including evaluation of transfer factor, limb salvaging, and evaluation of the time/therapy sequence in treatment of metastatic disease). Extensive investigation of new agents is required in high risk Wilms' and Ewing's sarcoma and rhabdomyosarcoma. Efficacy studies of

appropriate imaging techniques are mandated in virtually all protocols in which tumor delineation is essential. And in all studies active participation by nurse specialists should be encouraged in order to enhance protocol development.

3. MULTIPLE CHEMOTHERAPY

242. CLINICAL USE OF DDMP (2,-DIAMINO-5-(3',4'-DICHLOROPHENYL)-6-METHYL PYRIMIDINE) WITH CONCURRENT 'HIGH' DOSE FOLINIC ACID

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The protocol used of 'high' dose DDMP with concurrent 'high' dose folinic acid (CF) administered weekly was based on results from experimental laboratory studies (ICRF Scientific Report 1977 - report 118). The value of this concurrent combination has been described previously (ICRF Scientific Report 1978 - report 19) and an update of this ongoing trial is now provided, which emphasizes the lack of toxicity in this particular protocol.

Eighty patients with progressive malignant disease received DDMP (1-10 mg/kg) with simultaneous CF (1-8 mg/kg). In 75 assessable patients, 21 have achieved responses and 6 patients have disease arrest (all hypernephromas). Responses were seen in squamous cell carcinoma of the lung and larynx, melanomas, hypernephromas and one astrocytoma. In the course of 854 treatment cycles the platelets fell below 50,000/cu. mm on only 18 occasions and the total white count was reduced to less than 2,000/cu. mm only six times. CNS toxicity and gastrointestinal toxicity were minimal and there were no drug-induced deaths. This combination has therefore been shown to be effective and is well tolerated as an out-patient treatment. Other studies in North America and Europe have used different schedules, some of which have proved exceedingly toxic, especially those using bi-weekly DDMP or not administering CF. The use of CF as a 'rescue' 24 hours after DDMP has also resulted in far more toxicity than in our study using CF as a 'protection' agent.

243. CHILDREN'S CANCER STUDY GROUP

Newton, W. A., Miser, A., Miser, J., Distelhorst, C., Mulne, L., Rothermel, K., Boles, E. T., King, D., Clatworthy, H. W., Childrens Hospital, Dept. of Pathology/Pediatrics, *561 S. 17th St., Livingston Pike, Columbus, Ohio*, 43205, United States of America

This is a continuation of a cooperative inter-institutional study of cancer in children, utilizing multi-disciplinary approach to treatment of those types of cancer which are suited for scientific analysis in a reasonable period of time. The studies are designed to provide answers to questions concerning optimum management, and at the same time provide the individual institutions with the added strengths to supply their communities with a referral center for childhood cancer.

At the time of writing, there are 11 active protocols dealing with leukemia and 27 dealing with solid tumors, including Phase II studies. The principal protocol for ALL/AML is designed to determine the relative importance of front-end factors in prognosis, with randomized increased therapy during maintenance for those with known poor prognostic findings. The present AML protocol is an effort to improve survival and duration of remission in childhood AML by combining the two best therapies from CCSG 241 and CCSG 102.

Specific protocols currently being used are those for neuroblastoma, Stage III and IV; histiocytosis; retinoblastoma, unilateral and extraocular; brain tumors; osteogenic sarcoma; non-Hodgkin's lymphoma and ovarian tumors. On an intergroup basis, there are active protocols for rhabdomyosarcoma, Ewing's tumor both non-metastatic and metastatic, Hodgkin's disease and Wilms' tumor. Protocols being formulated and finalized are for bilateral retinoblastoma, brain tumors, nasopharyngeal carcinoma, and hepatoma.

In addition, ongoing Phase II protocols utilize a succession or combination of new therapeutic agents such as Vindesine, galactitol, Azapicyl, Prednimustine, beta-TGdR, cis-platinum, Poly I:Poly C and hexamethylmelamine.

BIBLIOGRAPHIC REFERENCES: Raney, R.B., Gehan, E.A., Maurer, H.W., Newton, W.A., Ragab, A.H., Ruymann, F.B., Sutow, W.W., and Tefft, M.: Evaluation of intensified chemotherapy in children with advanced rhabdomyosarcoma (Clinical Groups III and IV). Cancer Clinical Trials, 19-28, Spring, 1979.

244. STUDY AND TREATMENT OF CANCER IN THE YOUNG

Levine, A. S., Poplack, D. G., Glaubiger, D., Pizzo, P. A., Deisseroth, A. B., Magrath, I. T., Simon, R., Ziegler, J. L., Rosenberg, S. A., Glatstein, E., Chabner, B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Pediatric Oncology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Acute leukemia, non-Hodgkin's lymphomas (e.g., Burkitt's), neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma and aplastic anemia are studied. In the treatment of leukemia, we have devised therapeutic regimens which may allow maximum tumor cell kill while minimizing sequellae. Emphasis is on tailoring treatment to individual prognostic variables, and improving the therapeutic ratio in CNS prophylaxis. In treating the solid tumors, we have developed combined modality approaches to primary disease. In refractory tumors, we are studying the utility of high-dose-therapy and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, autologous bone marrow rescue, total parenteral nutrition) in permitting such therapy. Phase I-II trials are also conducted in refractory tumors; agents include ICRF-187, poly I:C/poly-l-lysine, dihydroxyanthracenedione, and 2'-deoxycoformycin. We are exploring the biology of selected tumors, including kinetics, immunology, virology, tumor markers, genetics, biochemistry, and pharmacology. Also studied are the effects of cancer and its treatment on growth, development, and organ function. The psychosocial concomitants of cancer are explored.

BIBLIOGRAPHIC REFERENCES: Glaubiger, D., Makuch, R., Schwarz, J., Levine, A.S., and Johnson, R.E.: Determination of prognostic factors and their influence on therapeutic results in patients with Ewing's sarcoma. Cancer 45: 2213-2219, 1980. Shackney, S.E., Bunn, P.A., Ford, S.S., Erickson, B., Ross, W.E., and Levine, A.S.: A study of drug-induced kinetic perturbations in the marrow of a patient with neuroblastoma. Cancer 45: 882-892, 1980.

245. SOUTHWEST ONCOLOGY GROUP (PEDIATRICS)

Van Eys, J., Cangir, A., Sutow, W. W., Sullivan, M. P., Jaffe, N., Ried, H. L., Culbert, S. J., Frankel, L. S., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Pediatrics, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas*, 77030, United States of America

Investigative clinical therapeutic studies in children with acute leukemia, malignant lymphoma, histiocytosis X, and malignant solid tumors (brain tumors, neuroblastoma, Wilms' tumor, osteogenic sarcoma, Ewing's sarcoma, soft tissue sarcoma, and miscellaneous malignant neoplasms) are conducted in four areas: (a) Phase I coordinated studies of human pharmacologic and toxicologic aspects of new anticancer agents and new combinations and dosage regimens; (b) Phase II systematic investigations of the effectiveness of individual anticancer agents or treatment programs in specific tumors; (c) Phase III comparative evaluations of selected drugs and/or therapy regimens; and (d) specific laboratory investigations into immunology and clinical pharmacology. The functioning cooperative group study mechanism is utilized to increase the size of the study populations and to shorten the duration of the study.

Independent investigative probes are undertaken to explore leads resulting from or directed toward group studies. Several intergroup and national studies, ongoing and projected (National Wilms' Tumor Study, Intergroup Rhabdomyosarcoma Study, Intergroup Ewing's Sarcoma Study, Hepatoma Study, Hodgkin's Disease Study) are included.

This research will be executed in an integrated multimodal manner. Attention will be given to centralized data management for the individual specialities of pediatrics, surgery, radiotherapy, pathology and laboratory support. Special attention will be given to clinical pharmacology and the immunological investigation required for optimal execution of current protocols employing high dose methotrexate or protocols requiring classifica-

tion of lymphoid malignancies in order to establish proper therapy assignment.

246. SOUTHWEST ONCOLOGY GROUP PROTOCOLS 7719, 7727/28, 7730

Reed, R. C., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *San Antonio, Texas*, 78284, United States of America

The objective of these protocols is to evaluate the efficacy of combination chemotherapy with adriamycin, BCNU, prednisone, vincristine, DDP and bleomycin for relapsing myeloma patients with BCNU, hydroxyurea and DTIC, with or without levamisole or DTIC and adriamycin-D in previously untreated patients with disseminated melanoma and with DDP in refractory disseminated melanoma patients. Previously untreated melanoma patients who have brain metastases are also given decadron and whole brain irradiation and those with liver metastases are given DTIC cannula into the hepatic artery. Patients on these protocols are examined at appropriate time intervals to assess tumor response, length of response, side effects and survival from start of treatment. These protocols are currently active in the Southwest Oncology Group. We have treated (O) patients with myeloma, (O) previously untreated disseminated melanoma patients and (O) relapsing disseminated melanoma patients with these protocols due to the low incidence of these diseases among our patient population.

247. CLINICAL CANCER RESEARCH CENTER

Burchenal, J. H., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York*, 10021, United States of America

The four drug sequence, cyclophosphamide, vincristine, F3TdR and papaverine, designated as the N-3, has proved effective in inducing remission in patients with disseminated neuroblastoma considered resistant to conventional and experimental drugs and radiation.

The drug administration program has undergone some evolution based upon the experience with the first twenty-eight patients. The number and intensity of courses required to totally eliminate the tumor, and further minimization of the morbidity following drug administration and tumor cell lysis remain to be established. The use of in vitro and in vivo (nude mice) tumor-drug sensitivity profiles for each patient has been shown to be feasible and their prospective value will be determined.

248. COMBINED CHEMOTHERAPY FOR METASTATIC NEUROBLASTOMA, STAGE IV (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

4. SURGERY, RADIOTHERAPY AND CHEMOTHERAPY

249. THERAPEUTIC CONTROLLED STUDY OF GLIOMAS OF PEDIATRIC AGE

Ambrosio, A., Colangelo, M., Palmieri, A., Catalano, E., Capece, W., Licenziati, F., Ospedali Riuniti per Hambini di Napoli, Dept. of Pediatric Neurosurgery, *Via Mario Fiore 8, 80131 Naples, Italy*

The main goal of the research consists of determining differences between two different combined treatments of the gliomas (Surgery plus Radiotherapy plus Chemotherapy versus Radiotherapy plus Surgery plus Chemotherapy). The evaluation will be made in terms of survival and a useful recovery.

Furthermore, the administration of radiation therapy and of chemotherapy, according to the usual dosages, will be critically estimated by studying their incidence on these parameters: Intracranial pressure (ICP), bioelectrical cerebral activity (EEG), and reduction in the volume of neoplasm.

In the case of pre-surgical irradiation, the approach is performed through the pre-operative evaluation, consisting of CT-scan, monitoring of ICP and EEG, followed by intraoperative observation of a certain number of facors, which are: 1) reduction in the volume of neoplasm, 2) reduction in the bleeding and a much easier hemostasis, 3) a much easier condition for removing the neoplasm from the surrounding area, and 4) a much easier fragmentation of the tumoral mass.

In the case of post-operative irradiation, the approach is made during the follow-up time and it employs the described procedure of evaluation (CT-scan, ICP and EEG monitoring) according to a scheduled program.

250. PROTOCOL OF TREATMENT FOR RETINOBLASTOMA USING SURGERY, RADIOTHERAPY AND CHEMOTHERAPY - PHASE III

Lobo, J. F., Guerrero, L. J., Piza, E. J., Camacho, M. A., Hospital Nacional de Ninos, *Apdo. 1654, San Jose, Costa Rica*

OBJECTIVE: To establish the usefulness of treatment comprised of surgery, radiotherapy and chemotherapy according to stage of tumor.

APPROACH: Determination of stage: Stage 1 - surgery; Stage 2 -chemotherapy for 1 year (vincristine plus cyclophosphamide), surgery; Stage 3 - surgery, radiotherapy and chemotherapy using vincristine, cyclophosphamide, methotrexate; Stage 4 - same as 3 plus radiotherapy to metastases. Controlled trial.

PROGRESS: Twenty-five patients under evaluation. (Translated)

251. PROTOCOL OF TREATMENT FOR NEUROBLASTOMAS USING SURGERY, RADIOTHERAPY, CHEMOTHERAPY - PHASE III

Lobo, J. F., Garcia, I., Vargas, G., Camacho, M. A., Carrillo, H. J., Jimenez, B. R., Jimenez, E., Navarrete, M., Mora, L., Hospital Nacional de Ninos, Dept. of Onco-Hematology, *Apdo. 1654, San Jose, Costa Rica*

OBJECTIVE: To improve survival through therapeutic approach in relation to age and extent of disease.

APPROACH: Under 1 year: Stages I and II: resection and chemotherapy; Stages III and IV: chemotherapy (vincristine, cyclophosphamide, adriamycin). Over 1 year: Stages I and II: resection, chemotherapy, radiotherapy. Stage III: chemotherapy, radiotherapy with 60Co. Stage IV: chemotherapy. Controlled trial.

PROGRESS: Twenty-five cases under study. (Translated)

252. GLIOBLASTOMA MULTIFORME IN THE ADULT - SURGERY, IRRADIATION AND CYTOSTATICS

Jimenez Medina, J., Alert, J., Nicot, C., Alfonso, L., Verdecia, N., Ministerio de Salud Publica, Instituto Nacional de Oncologia y Radiobiologia, Departamento de Radioterapia, *Calle 29 y E Vedado, Havana 4, Cuba*

OBJECTIVE: To increase survival of patients with gliblastoma multiforme, which averages 6 months in Cuba.

METHOD: To combine surgery and ionizing radiation with various cytostatics administered orally and intrathecally.

253. BRAIN TUMOR PROTOCOL 77-02 - SURGERY, RADIATION THERAPY, BCNU, RADIATION SENSITIZER, AND STREPTOZOTOCIN

Cashion, E. L., Robertson, J. T., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Neurosurgical Section, *1030 Jefferson Ave., Memphis, Tennessee*, 38104, United States of America

The purpose of this study is to determine, in a patient population with known malignant glioma who have undergone cytoreductive surgery and in a controlled prospective randomized study, the efficacy of: (1) Superfractionated radiotherapy and BCNU; (2) radiotherapy and the radiosensitizer Misonidazole followed by BCNU; (3) radiotherapy and Streptozotocin; (4) BCNU and radiotherapy.

At the present time, 86 patients have been randomized to this study by the University of Tennessee. There have been 34 deaths from tumor progression and four deaths from secondary medical problems.

Seven Veterans Administration patients have been randomized to the study and five are still receiving treatment.

This protocol is anticipated to complete patient accrual by October 1, 1980.

We are observing a longer survival in the patients being treated on the study. Through experience, we have been able to more effectively control the immediate toxicity to the chemotherapeutic agents by the use of specific antiemetic drugs. We are

observing a greater amount of myelosuppression in the hematologic monitoring of patients treated than we have experienced in the past. There have been no major complications because of myelosuppression.

254. SOUTHWEST ONCOLOGY STUDY GROUP

Crist, W. M., Castleberry, R. P., Dearth, J. C., Parmley, R. T., Salter, M., Cooper, M., Lawton, A., Bueschen, A., Buntain, W., University of Alabama in Birmingham, School of Medicine, Dept. of Pediatrics, *1919 7th Ave. S., Birmingham, Alabama,* 35233, United States of America

Support is requested to permit continued participation in clinical and laboratory investigative activities in the Southwest Oncology Group. Specifically these funds will assist in support of our effort in cooperative cancer therapy trials which utilize a combination of surgery, and/or radiation therapy, and/or chemotherapy for the investigative clinical trials of leukemias, lymphomas, and a variety of childhood solid tumors. Emphasis will be placed on integrated multi-modality treatment programs in Phase III randomized prospective studies. We are also participating in several pilot studies of new drug treatment programs, in Phase II trials, and non-randomized studies.

Additionally, continued support for immunologic evaluation of all new acute lymphocytic leukemia patients in the pediatric division of SWOG is requested. Specifically, cytoplasmic staining (characteristic of pr-B cells) of lymphoblasts prepared on slides and shipped to us is being accomplished efficiently as an ongoing groupwide project, in order to better characterize and classify lymphoid malignancies.

Support is also requested for two new projects. The first will examine the role of HLA typing (especially B lymphocyte antigen typing) in predicting risk for or resistance to malignancy and also relationships to survival duration once malignancy is established. A second project will establish the incidence of circulating immune complexes in neuroblastoma, acute lymphoblastic leukemia, and osteogenic sarcoma and their correlation with disease activity.

255. SURGERY, RADIATION THERAPY AND CHEMOTHERAPY OF BRAIN TUMORS

Ransohoff, J., Foo, S., Feigin, I., Newall, J., Cravioto, H., George, A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *1st Ave. at E. 24th St., New York, New York,* 10010, United States of America

Between October 1, 1979 and September 30, 1980, New York University Medical Center randomized a total of 65 patients into the three national Brain Tumor Study Group protocols open to accrual during this fiscal year. These are multidisciplinary protocols involving surgery, radiation therapy, and chemotherapy. Nineteen patients entered Phase III 77-02 and 13 patients were randomized to Protocol 78-20. There were no suitable candidates for Phase III Protocol 77-01. VA participation was as follows: one patient received treatment under Protocol 77-02 and 13 patients were randomized to Protocol 78-20. One patient was randomized at Bellevue Hospital but was treated at the VA facility. During this period, we encountered no new serious side effects of the drugs presently being utilized. A statistical analysis on the survivial of patients in BTSG protocols should be made available to the National Cancer Institute shortly. We plan to continue evaluating patients in the NCI protocols including the VA participation. New protocols are being formulated and will be submitted for approval in the near future.

256. STUDY OF THE TREATMENT OF MALIGNANT GLIOMA OF THE BRAIN

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The Brain Tumor Study Group is the National Cancer Institute's prime source of treatment testing for compounds and approaches which have a high potential for the treatment of brain cancer.

Phase II BTSG protocols are designed to admit patients with brain tumors not eligible for the Phase III study in order to determine the comparative value of a given mode of therapy, the problems in utilizing such therapy, and the toxicity which might be reasonably anticipated. The basic design is one of a continuous flow protocol in which specific agents will be replaced in the study as their comparative value is determined. The protocol will allow for a more careful and rapid selection of potential agents for Phase III studies.

The current randomized Phase III studies are: Surgery followed by Radiotherapy and BCNU vs. Radiotherapy and BCNU followed by Surgery; Surgery, Radiation Therapy and BCNU vs. Surgery, Fractionated Radiotherapy and BCNU vs. Surgery, Radiation Sensitizer and Radiation Therapy followed by BCNU vs. Surgery, Radiation Therapy and Streptozotocin.

257. TUMORS OF THE NERVOUS SYSTEM

Ommaya, A. K., Walters, C., Reed, J., Kochie, P., Chretien, P., Poplack, D., Sadowsky, D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Neurological & Communicative Dis. & Stroke, Section on Applied Research, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

A clinical study of intratumoral chemotherapy using 8-azaguanine compares the effect of this method given in combination with systemic CCNU after surgery and radiotherapy with the effect of adjuvant chemotherapy with CCNU given alone. In a pilot study, our methods have increased the mean survival time to approximately twice that reported by other centers using CCNU alone after surgery and radiotherapy. Dosage of therapeutic agents used for local intrathecal chemotherapy is being monitored in a primate model for chronic intrathecal chemotherapy. Our immunologic approach is currently embodied in a Phase I study of treatment with thymosin. Based on in vitro studies with thymosin in our gliomas patients we have completed a Phase I study comparing the in vitro effects of thymosin V and thymosin alpha 1 in 14 patients. Further work has been stopped due to administrative interference.

5. NUTRITIONAL THERAPY AND SUPPORT

258. PREVENTION AND REVERSAL OF MALNUTRITION IN NEUROBLASTOMA

Baehner, R. L., Rickard, K. A., Grosfeld, J. L., Matchett, N., Weetman, R. M., Provisor, A. J., Boxer, L. A., Oei, T. O., Indiana University - Purdue University at Indianapolis, School of Medicine, Dept. of Pediatrics, *1219 W. Michigan St., Indianapolis, Indiana,* 46202, United States of America

The prognosis for stage IV children with neuroblastoma is dismal. Aggressive multimodal treatment programs often result in severe malnutrition (PEM) which interferes with therapy and increases morbidity and mortality. The plan of this study is to evaluate the effectiveness of enteral and parenteral nutrition in preventing and/or reversing PEM, maintaining or restoring immune competence, preventing treatment delays, and improving tumor response of 36 children (ages one to six years) with metastatic neuroblastoma receiving chemotherapy (5 drugs: Cytoxan, vincristine, DTIC, adriamycin, VM-26) every three weeks. At diagnosis, children who are considered nourished at diagnosis will be randomized to receive either a comprehensive enteral nutrition program or total parenteral nutrition (TPN) for the first 35 days of treatment. Children considered malnourished at diagnosis will be randomized to receive either TPN or partial parenteral nutrition plus enteral nutrition for the first 35 days of the treatment. Thereafter, all children will receive a comprehensive enteral nutrition program. Nutritional status, recall skin test response, treatment delays and tumor response will be evaluated at 0, 6, 12, 18 and 22 weeks. Additional nutritional evaluations (dietary, anthropometric and biochemical) will be determined weekly during the first 5 weeks to identify nutritional indices of clinical and subclinical PEM in children undergoing treatment for metastatic neuroblastoma.

259. STUDIES OF DIFFERENTIAL NUTRITIONAL REQUIREMENTS BY HOST

Robertson, J. T., University of Tennessee, Center for the Health Sciences, School of Medicine, Dept. of Neurosurgery, *62 S. Dunlap St., Memphis, Tennessee,* 38103, United States of America

OBJECTIVE: Perform research on cancer patients with malignant brain tumors for the clinical segment of this project. During and after the experimental period, patients will be followed and evaluated to assess their response to direct nutritional therapy. In vivo studies using animal models and in vitro studies

will be coordinated with the clinical segment. Experimental protocols and data collection for this project will be coordinated with another similar project to be sponsored by the DNCP. The principal investigator will be expected to preserve and/or make available biological samples and specimens for possible collaborative study by other scientists.

260. DIETARY TREATMENT OF BRAIN TUMORS (HUMAN)

Banks, W. L., Virginia Commonwealth University, Medical College of Virginia, Dept. of Biochemistry/Surgery, *1200 E. Broad St., Richmond, Virginia*, 23298, United States of America

This is part of a broader project. A summary of this subproject is not available.

261. POTENTIAL OF HOME TOTAL PARENTERAL NUTRITION DURING CANCER CHEMOTHERAPY

Burchenal, J. H., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

C. CANCER TREATMENT STUDY GROUPS

262. CANCER CENTER SUPPORT GRANT (SPECIALIZED)

Camitta, B. M., Medical College of Wisconsin, School of Medicine, Dept. of Pediatrics, *561 N. 15th St., Milwaukee, Wisconsin*, 53233, United States of America

The diagnosis of cancer in a child is no longer synonymous with death. However, cancer is still the leading cause of death from disease in American children over one year of age. In addition, most of the major advances in treatment were made prior to 1970 and the dismal prognoses of some tumors (T cell leukemia, disseminated neuroblastoma) remain unchanged. Even in therapy-responsive tumors such as common cell ALL, lymphomas and Wilms' tumor, 20-50% of children relapse. Clearly there is a need for more fundamental knowledge about the origin, biologic properties, subtypes, and reasons for varying responsiveness to therapy of different childhood tumors.

The Midwest Children's Cancer Center is a clinical research facility, associated with research laboratories focusing on problems generated by patient care. The six general objectives of the Center are: (1) Determination of pharmacokinetics, mechanisms of action and efficacy of new anti-cancer drugs; (2) development of further understanding of the immune system in children with cancer and possible ways of utilizing it for treatment; (3) development of new anti-cancer treatments by comparative and exploratory studies; (4) assessment of infectious problems in children with cancer and ways of treating or preventing them; (5) provision of childhood cancer education for physicians, basic scientists, students, nurses, and the public; (6) collaboration with neighboring institutions, other Cancer Centers and community programs.

On a larger scale, the Cancer Center's aim is to generate awareness, interest and involvement in childhood cancer by the medical community and lay public. It also serves as a model for organization of care and research for other life threatening diseases requiring intensive long-term multidisciplinary treatment.

BIBLIOGRAPHIC REFERENCES: Pinkel, D., Hustu, H.O., Aur, R.J.A., Smith, K., Borella, L., and Simone, J.: Radiotherapy in leukemia and lymphoma of children. Cancer, 39:817, 1977. Borella, L., Sen, L., Dow, L.W., and Casper, J.T.: Cell differentiation versus tumor-related antigens in childhood acute lymphoblastic leukemia (ALL). Clinical significance of leukemia markers. Haematology and Blood Transfusion 20:77-85, 1977.

263. CHILDREN'S CANCER STUDY GROUP

Heyn, R. M., Ertel, I., Hutchinson, R. J., Hanash, S., Holland, R., Heidelberger, K., Schnitzer, B., Waskerwitz, M., Hanson, T., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Pediatrics & Communicable Diseases, *1335 E. Catherine St., Ann Arbor, Michigan*, 48104, United States of America

The Children's Cancer Study Group is engaged in carrying out clinical research studies which have been developed by senior investigators in the group and approved by the NCI. These programs, which are designed to improve the care and survival of children with leukemia and solid tumors, include both Phase II and Phase III studies. Phase II studies are primarily those involving new agents which have completed Phase I trial.

The current study for children with newly diagnosed acute lymphocytic leukemia involves stratification at diagnosis based on white blood count, age, and cell morphology. Remission is induced with prednisone, vincristine and L-asparaginase in all children and similar CNS prophylaxis is given to all except the good-risk group which is randomized to either intrathecal methotrexate or cranial irradiation and intrathecal methotrexate. All patients are then randomized for various maintenance programs to answer the question of whether it is advantageous to 1) give further intermittent intrathecal methotrexate and 2) various intermittent additive drugs to systemic chemotherapy with daily 6-mercaptopurine and weekly methotrexate orally.

Children with acute non-lymphocytic leukemia are induced with continuous infusion cytosine arabinoside for 7 days plus 3 daily doses of adriamycin, given at 2 week intervals. If remission is obtained, children with HLA sibling matches will receive bone marrow transplant. Those without such matches will enter a chemotherapy maintenance program involving cyclic versus continuous use of multiple agents.

Solid tumor studies include the intergroup studies for Wilms' tumor, Ewing's sarcoma and rhabdomyosarcoma. Studies for Stage I and II Hodgkin's disease and non-Hodgkin's lymphoma are being done in conjunction with other cooperative groups. Other Children's Cancer Study Group studies are current for brain tumors, osteogenic sarcoma, histiocytosis X, retinoblastoma, liver tumors and ovarian tumors.

BIBLIOGRAPHIC REFERENCES: Heidelberger, K.P., Schnitzer, B., Tilford, D., Poznanski, A.K., and Heyn, R. Marked skeletal involvement in sinus histiocytosis with massive lymphadenopathy. Accepted for publication in Skeletal Radiology, May, 1979. Lahey, M.E., Heyn, R.M., Newton, W.A. Jr., Shore, N., Smith, W.B., Leikin, S., and Hammond, D. Histiocytosis X: Clinical trial of Chlorambucil. A report from Children's Cancer Study Group. Accepted for publication in Medical and Pediatric Oncology, 1979.

264. SOUTHWEST ONCOLOGY GROUP, PEDIATRIC DIVISION

Pullen, D. J., Miller, R. C., Hickman, B. T., Johnson, W. W., Iyer, R. V., Jackson, J. F., Routh, A., University of Mississippi, Medical Center, School of Medicine, Dept. of Pediatrics, *2500 N. State St., Jackson, Mississippi*, 39216, United States of America

The University of Mississippi Medical Center (U.M.C.) Department of Pediatrics has participated in the treatment protocols and activities of the Southwest Oncology Group (SWOG) since July 1970. In addition to the U.M.C. Division of Pediatric Oncology, the disciplines of pediatric surgery, pediatric pathology and radiation therapy are active participants in the program, as are supportive specialties including pediatric radiology, immunology and medical genetics.

The Southwest Oncology Group Pediatric Divisions' central objective is the delineation of increasingly effective means for treating various types of childhood malignancies utilizing the combined efforts of the participating institutions in protocol design and implementation. These collaborative studies form the basis of the U.M.C. Department of Pediatrics' clinical research program in oncology. Studies are currently in progress for acute lymphocytic leukemia, acute granulocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, histiocytosis X, Wilms' tumor, neuroblastoma, rhabdomyosarcoma, osteogenic sarcoma, Ewing's sarcoma, brain tumor and malignant teratoma.

In addition to participating in the SWOG clinical research studies, the U.M.C. disciplines are active in committee functions and protocol development. The U.M.C. Department of Pediatrics is serving as the coordinating institution for the SWOG AlinC 13 pilot and final classification and treatment protocols. ALinC 13 will be the next SWOG pediatric front-line study for acute lymphocytic leukemia.

BIBLIOGRAPHIC REFERENCES: Pullen, J., Crist, W., Falletta, J., Humphrey, B., Metzgar, R., van Eys, J., Vogler, L.: Immunologic profiles in childhood acute lymphocytic leukemia (ALL). Proc AACR 21:247, 1980. Frankel, L.A., Wang, Y.M., Pullen, J., Nitschke, R., Doering, E.J.: High dose intravenous

(IV) methotrexate (MTX) with an intrathecal (IT) boost in the maintenance of acute lymphocytic leukemia (ALL) in children. Proc ASCO 21:389, 1980.

265. CHILDREN'S CANCER STUDY GROUP

Miller, D. R., Steinherz, P. G., Allen, J. C., Tan, C., Memorial Hospital for Cancer & Allied Diseases, Dept. of Pediatrics, *1275 York Ave., New York, New York*, 10021, United States of America

The Children's Cancer Study Group is a multidisciplinary cooperative group of investigators from 26 institutions in the United States and Canada collaborating on the development, implementation and completion of prospective, randomized clinical trials in pediatric hematologic malignancies and solid tumors, including acute lymphoblastic leukemia (ALL), acute nonlymphoblastic leukemia (ANLL), brain tumors, lymphomas (non-Hodgkin's and Hodgkin's), Wilms' tumor, neuroblastoma, rhabdomyosarcoma, bone tumors, hepatoblastoma, 'histiocytosis X', and retinoblastoma. Natural history, biologic characterization of cancer (pathology, immunology, biochemistry), prognostic factors, late effects and supportive care aspects are also stressed in these studies involving pediatric oncohematologists, surgeons, pathologists, diagnostic and therapeutic radiologists, neurologists, psychiatrists, psychologists, cell biologists, immunologists, and infectious disease specialists. New agent Phase II trials for patients failing initial therapy and new modalities of therapy, including bone marrow transplantation, are additional major thrusts of the cooperative group. MSKCC - NYH-CMC (and its affiliates) has been a major contributor to the efforts of the Group. This research proposal outlines the center's participation in present and future CCSG endeavors at the executive level as well as in the development of new pilot studies for eventual groupwide participation.

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266. SOUTHWEST ONCOLOGY GROUP

Baker, L. H., Wayne State University, School of Medicine, Dept. of Oncology, *1014 Rivard, Detroit, Michigan*, 48207, United States of America

Wayne State University is the leading contributor of patients to Southwest Oncology Group protocols. In five of the thirteen committees of the group, Wayne State has contributed ten percent or more of the total population of patients studied by that group. These committees include: Brain (22%), GI (12%), GYN (10%), Lung (10%), Sarcoma (13%).

Wayne State University is represented on each disease committee of the adult division by member medical oncologists, pathologists, surgeons or radiation therapists.

Members of the department are chairing (or co-chairing) the following major southwest studies: Dr. Samson, 7817 testicular study; Dr. Baker, 7613 sarcoma study; Dr. Al-Sarraf, 7727/28 melanoma study; Dr. Samal, 7725 breast study; Dr. Buroker, new first line Phase II-III large bowel study. Other Phase II studies being chaired by faculty members include: Dr. Samson, 7611 Platinum in Sarcomas; Dr. Voravit, 7821 Maytansine in Lymphomas; Dr. Al-Sarraf, 7730 Platinum in Melanoma and 7716 Tamoxifen in Renal Cancer; Dr. Baker, 7870 Mitomycin and Bleomycin in Cervical Cancer.

The WSU faculty hold leadership roles in Southwest Oncology Group administration. Dr. Vaitkevicius and Dr. Buroker are Chairman and Vice-Chairman of the GI Committee, Dr. Baker is Chairman of the Sarcoma Committee, a member of the Executive Committee of the Adult Division, serves on the Steering Committee of GYN Committee and is a representative to the Pharmacology Committee.

BIBLIOGRAPHIC REFERENCES: Baker, L.H., Opipari, M.I., Wilson, H., Bottomley, R., and Coltman Jr., C.A. Mitomycin C, Vincristine and Bleomycin Therapy for Advanced Cervical Cancer. Obstetrics and Gynecology, 52: 146-150, 1978. Benjamin, R.S., Baker, L.H., O'Bryan, R.M., Moon, T.E., and Gottlieb, J.A. Chemotherapy for Metastatic Osteosarcoma - Studies by the M.D. Anderson Hospital and the Southwest Oncology Group. Cancer Treatment Reports 62: 237-238, 1978.

267. MULTIDISCIPLINARY STUDIES OF CHILDHOOD CANCER - SWOG

Maurer, H. M., McWilliams, N. B., Russell, E. C., Dunn, N. L., Hazra, T. A., Lawrence, W., Salzberg, A., Kay, S., Mohanakumar, T., Snead, R. W., Virginia Commonwealth University, Medical College of Virginia, Dept. of Pediatrics, *1200 E. Broad St., Richmond, Virginia*, 23298, United States of America

The multidisciplinary team of pediatric oncology investigators at the Medical College of Virginia will: (1) participate collaboratively with Southwest Oncology Group in cooperative programs to determine the optimal treatment regimens for infants, children and adolescents with leukemia and solid tumors; (2) continue to provide leadership in treatment of childhood rhabdomyosarcoma through the Intergroup Rhabdomyosarcoma Study of CALGB, CCSG and SWOG; (3) provide leadership in neuroblastoma and sarcoma studies; (4) develop studies of late effects of cancer therapy in children; (5) develop and participate in SWOG pilot studies in childhood cancer; (6) provide leadership in surface marker and other immunological studies; (7) collect precise data by high quality performance and adherence to protocol procedures and record-keeping; (8) participate in modality and disease-oriented subcommittees of SWOG to develop multidisciplinary studies of therapy for childhood cancer.

BIBLIOGRAPHIC REFERENCES: Maurer, H.M., (for the IRS Committee): The Intergroup Rhabdomyosarcoma Study. II: Objectives and study design. J. Pediatr. Surg. 15:371, 1980. (Maurer, H.M., Chairman IRS Committee), Tefft, M., Hays, D., Raney, R.B., Lawrence, W., Soule, E., Donaldson, M.H., Sutow, W.W., Gehan, E.: Radiation to regional nodes for rhabdomyosarcoma of the genitourinary tract in children: Is it necessary? Cancer 45:3066, 1980.

268. SOUTHWEST ONCOLOGY GROUP

Humphrey, G. B., Nitschke, R., Sexauer, C. L., Blackstock, R., Altshuler, G., Krous, H. F., Altmiller, D. H., Bogardus, C. R., Maxwell, J. D., University of Oklahoma, Health Sciences Center, School of Medicine, Dept. of Pediatrics, *Box 26901, Oklahoma City, Oklahoma*, 73190, United States of America

This proposal involves a multi-disciplinary/multi-professional research approach to childhood malignancies. This proposal is supported by a clinical research and laboratory research team, and is actively involved with the Pediatric Division of the Southwest Oncology Group (SWOG). Special emphasis is given to the evaluation of curative therapies to increase effectiveness and/or eliminate toxicity or unnecessary components. Oklahoma is actively involved in pilot studies for SWOG that are designed to improve the treatment in noncurative cases. Oklahoma is also involved in ancillary studies in compliance, pain control, etc., that could also influence future group studies. Through its Immunology Laboratory based in pediatric oncology, we have developed standard methodological protocols for the Pediatric Division so that surface markers in acute lymphocytic leukemia can be used on a group-wide basis. Through the Pathology Laboratory, group-wide determination of catecholamines for neuroblastoma is proposed. Members of the oncology team are involved in administrative and scientific committees of SWOG in hematopoietic diseases, neuroblastoma, immunology, solid tumors, pathology, surgery, cancer control, and administrative review.

269. SOUTHWEST ONCOLOGY GROUP - PEDIATRICS

Leventhal, B. G., Kaizer, H., Civin, C. I., Haller, A. J., Wharam, M. D., Zinkham, W., Quddus, F. F., Johns Hopkins University, School of Medicine, Dept. of Pediatrics, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

Participation of senior oncology faculty in the Pediatric Oncology Group. It is proposed that the senior faculty will continue their participation in this group with assistance particularly in the design of new protocols for non-lymphocytic leukemia, bone marrow transplantation, double half body in rhabdomyosarcoma and neuroblastoma.

Patient accrual and participation in group protocols will continue at a vigorous level.

The glucocorticoid receptor laboratory, which has been organized and has begun to function, will begin correlation of

receptor studies with other clinical features of the leukemia cell studies as measured in other laboratories.

270. NORTHERN CALIFORNIA ONCOLOGY GROUP

Carter, S. K., Hannigan, J., Kaplan, M. J., Kohler, M., Brown, B., Friedman, M. A., Ray, G., Meurk, M. L., McGuigan, J. E., Northern California Cancer Program Inc., *770 Welch Rd., Suite 190, Palo Alto, California,* 94304, United States of America

The Northern California Oncology Group (NCOG), a clinical research activity of the Northern California Cancer Program (NCCP), is a cooperative clinical trials group which conducts multi-disciplinary clinical research projects dealing with a wide range of human malignancies. The NCOG consists of a mix of university oncology centers, restricted within the geographic region of NCCP which includes Northern California and Northwestern Nevada. The major attributes of NCOG are 1) regional, cancer center based, membership, 2) co-equal representation of all therapeutic modalities, 3) disease orientation, and 4) community outreach.

Twenty-two protocols are now activated, including pilots. Protocols cover lung, genitourinary, head and neck, CNS, breast, gastrointestinal, cervix, ovary, leukemia, melanoma and lymphoma cancers.

This application requests support for the 03, 04, and 05 years. The number of implemented groupwide and pilot protocols planned for the 03 year is 37 and 45 each for 04 and 05.

271. NCOG EDUCATION/DEMONSTRATION PROGRAM FOR COMMUNITY HEALTH PROVIDERS

Carter, S. K., Gottfried, D. F., Jackson, P., Sibary, K., Hannigan, J. F., Adams, F., Northern California Cancer Program Inc., *770 Welch Rd., Suite 190, Palo Alto, California,* 94304, United States of America

The overall goal of the NCOG Community Outreach Program is to assure the optimal diagnostic evaluation, staging, treatment, and rehabilitation of cancer patients in the participating Northern California Cancer Program regions. Two major programmatic thrusts are being developed toward this goal. First, multidisciplinary clinical committees were formed in each of the five participating regions: Greater Fresno, Sacramento, Greater Reno, the South Bay and the East Bay. Secondly, a broad range of professional education programs are being developed for physicians, nurses, pharmacists, and other medical professionals to complement the protocols and increase the individual's awareness of the most current diagostic treatment and rehabilitation strategies and techniques.

Professional education programs developed and implemented during the -01 year of the Outreach grant include: a symposium on genitourinary cancer, a discussion of primary surgery vs. primary radiation therapy for early stage breast cancer, a symposium on diagnosis, treatment and management of primary and metastatic brain tumors, and a discussion of the classification and grading of tumors of the testes and prostate.

272. CLINICAL RESEARCH PARTICIPATION IN NCOG

Friedman, M. A., Wilson, C. B., Phillips, T. L., Shohet, S. B., Churg, A. M., University of California, San Francisco Campus, School of Medicine, Dept. of Medicine, *551 Parnassus Ave., San Francisco, California,* 94122, United States of America

This new proposal presents the coherent clinical oncology activities at the University of California, San Francisco and its participation in the Northern California Oncology Group. Over the past year we have demonstrated an ability to perform as a coordinated, multidisciplinary force as a university and in this cooperative group activity. Recognizing the very modest level of funding afforded in the first year of our grant proposal, we have demonstrated the ability to provide clinical data of excellent quality and quantity. Particularly noteworthy have been the following activities: 1. We have provided the largest single institutional patient input to all clinical studies in the Northern California Oncology Group program (118 patients as of 8/78). 2. We have demonstrated a major scientific ability in the proposing of new studies for the group (including 5 Phase III studies, 6 Phase II studies, and 1 Phase I study). 3. We have demonstrated multidisciplinary cooperative activities with 2 plus modalities in 4 studies. 4. We have utilized our expertise in Clinical Pharmacology and Pharmacokinetics (Misonidazole and PCNU studies). 5. We have had a major leadership role in the NCOG with participation in all committees (Group Executive Officer, Chairmanships of the Surgery and Radiation Therapy Committee as well as 11 other Committees). 6. We have designed, developed, and streamlined the data management and statistical information records, designed data manuals and training facilities, and provided criticism of protocols.

We have been a major resource for patients and science in the area of gastrointestinal, breast, central nervous system, lung, and head and neck malignancies. We expect over the next several years to enhance and improve these activities. UCSF is a unique resource of demonstrated ability.

273. CHILDREN'S CANCER STUDY GROUP

Chard, R. L., Johnson, F. L., Pendergrass, T. W., Childrens Orthopedic Hospital & Medical Center, Division of Hematology/Oncology, *4800 Sand Point Way N.E., Box C-5371, Seattle, Washington,* 98105, United States of America

This proposal is for continuation of research into the natural history, treatment and epidemiology of the statistical conduct, design, collation, and publication of childhood cancer. It will continue to emphasize multimodal aspects of the Children's Cancer Study Group which has realized such excellent results in the past in Wilms' tumors and rhabdomyosarcoma and has now helped in defining acute childhood lymphocytic leukemia as more than one disease entity which requires different treatments for different groups. Also, treatment has been improved in osteogenic sarcoma and Ewing's sarcoma and, though advances are more meager, there have also been statistical improvements in survival of neuroblastoma patients. Childhood nonlymphocytic leukemia patients have also shown improved survival and the Children's Cancer Study Group has now combined in a Groupwide study to compare bone marrow transplantation in first remission to more standard conventional chemotherapy in a controlled fashion.

During the period from November, 1976 to December, 1979, 389 children with childhood cancer were seen at this institution; 182 with leukemia, 148 who were treated on study, 44 who were not on study, and 207 children with solid tumors. We have been a member of the Children's Cancer Study Group since 1959 and strong permanent referral patterns have been established. It is therefore anticipated that the patient load will continue to be stabilized and slightly increased over the next several years. A close working relationship with the adult leukemia center under Dr. E. Donnall Thomas at the Fred Hutchinson Cancer Research Center continues. This includes the exchange of professional personnel on the Fellowship level. Forty-three bone marrow transplantations on children with acute leukemia have been done at this institution from 11/1/76 to 12/1/79. Collaborative studies continue in immunology with the Fred Hutchinson Cancer Research Center, primarily through the efforts of Irwin D. Bernstein, M.D.

BIBLIOGRAPHIC REFERENCES: Bleyer, W.A., Krivit, W., Chard, R.L., Hammond, D.: Phase II study of VM-26 in acute leukemia, neuroblastoma and other refractory childhood malignancies. Cancer Treat. Rep. Vol. 63 6:977-981, 1979. Nesbit, M., Chard, R., Evans, A., Karon, M., Hammond, G.D.: Evaluation of intramuscular versus intravenous administration of L-asparaginase in childhood leukemia. Am. J. Pediatr. Hematology/Oncology Vol. 1, 1:9-13, 1979.

274. OSU COMPREHENSIVE CANCER CENTER SUPPORT (CORE) GRANT

Yohn, D. S., Cavalaris, C., Grever, M., Balcerzak, S., Glaser, R., Hart, R., Koestner, A., MacVicar, M., Ohio State University, Columbus Campus, School of Veterinary Medicine, Dept. of Veterinary Pathology/Comprehensive Cancer Center, *1900 Coffey Rd., Columbus, Ohio,* 43210, United States of America

The Ohio State University Comprehensive Cancer Center (OSUCCC) requests renewal for five (5) years of its Cancer Center (Core) Support Grant (CCSG). The CCSG will provide the necessary financial support that will assure quality administration of major programs in interdisciplinary basic and clinical cancer research, cancer education and training, cancer diagnosis and therapy, and community outreach. It is the intent of the OSUCCC to translate its own and other laboratories' observations into applicable clinical usage and to communicate existing and newly developed therapies, diagnostic procedures and prevention methods to the Ohio Valley region. The CCSG will provide salary support for directors of vital programs and for

key investigators necessary for further Center development. These individuals will collectively help to (1) optimally utilize and integrate the cancer efforts extant within the seven colleges at OSU engaged in cancer research; (2) develop new programs, pilot studies and demonstration projects in cancer research, education, diagnosis and therapy within the OSUCCC and at community levels; (3) encourage, aid, support and integrate community-run programs which are aimed to meet the needs of the respective areas; (4) develop interdisciplinary activities between clinical and basic scientists to affect the translation process from laboratory to clinical application; (5) communicate approved new developments in cancer diagnosis, therapy and rehabilitation to cooperating institutions throughout the Ohio Valley region; and (6) establish effective cooperation with other existing and developing cancer centers including the National Cancer Institute. The OSUCCC will undergo significant development during the next five years that should culminate in solidification of research programs and development of an effective cancer control program for the region.

275. CHILDREN'S CANCER STUDY GROUP

Piomelli, S., Sitarz, A. L., Wolff, J. A., Weiner, M., Vora, S., Santulli, T. V., Chang, C. H., Blanc, W. A., Berdron, W. E., Columbia University, College of Physicians & Surgeons, Dept. of Pediatrics, *630 W. 168th St., New York, New York*, 10032, United States of America

The Division of Pediatric Hematology of Babies' Hospital (the Pediatric Section of the College of Physicians and Surgeons of Columbia University) has participated in the research activities of Children's Cancer Study Group since 1957. Patients have been and will continue to be entered in the clinical trials of this Group, directed at devising optimal therapy for children's cancer and evaluating biological and natural history factors which are important; a multidisciplinary approach to cancer therapy will be followed, both at the institutional and at a national group level. Children with cancer cared for by members of the Division will be entered in the studies of the Group: they will receive medical care standardized to Group requirements, including all required laboratory and diagnostic investigations; accurate records will be kept and transmitted promptly to Group Headquarters for evaluation. Members of the Division will actively participate in the development of the research strategy of new studies. They will participate in the development of the Group scientific program, development and conduct of protocols for Ewing's sarcoma, retinoblastoma and non-Hodgkin's lymphoma, improved modalities for supportive care and definition of biological aspects of childhood cancer, refinement of Pediatric Surgical Oncology techniques and cost effective imaging techniques and coordination of the Group Pathology activities.

The Division of Pediatric Hematology/Oncology will coordinate the clinical care of childhood cancer in the Columbia Medical Community. Related research activities within the Division will be developed and eventually expanded to Group trials. These presently include studies of biochemical markers of malignancy; means to decrease toxicity of chemotherapy; the relationship between nutrition, cancer and chemotherapy; newly developed varicella vaccine and epidemiological aspects of perinatal cancer. The interaction between the clinical activities of the Division of Pediatric Hematology/Oncology and the comprehensive Cancer Center of Columbia University will be further developed.

276. CANCER CENTER SUPPORT GRANT

Cooper, R. A., University of Pennsylvania, School of Medicine, Dept. of Medicine, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

The University of Pennsylvania Cancer Center (UPCC) study is a multidisciplinary program in cancer research, education and clinical care. Based in the School of Medicine, it involves faculty from many schools of the University. The UPCC is one component of the Fox Chase-U. of P. Comprehensive Cancer Center. Interdisciplinary clinical research programs of the UPCC exist in hemotology-oncology, radiation therapy, surgical oncology and gynecologic oncology. A multidisciplinary program has been established in head and neck tumors, and similar programs are developing in neuro-oncology and urologic cancer. Clinical research programs utilize the shared resources of the Center's inpatient Oncology Study Unit, Clinical Trials Data Center, Pharmacology Core and Nutrition Support Service. Interdisciplinary fundamental research programs exist in immunobiology, cell biology, virology, genetics, and radiobiology. Program development involving tumor immunology and bone marrow culture is planned. Fundamental research programs utilize the shared resources of an animal breeding colony and tissue culture core, and they are strengthened by a Visiting Scientist Program. Acquisition of a fluorescence activated cell sorter is also planned. The Center's Epidemiology and Statistics Unit includes epidemiologic studies of occupational carcinogenesis and biostatistical research. The Unit established a biostatistics shared resource for the UPCC and a patient data system compatible with the CCPDS. A multidisciplinary Psychosocial Program, recently established, addresses needs in areas of research, service and education. Other Educational Programs have been established as well, and the UPCC provides a range of opportunities for medical and nursing students, fellows, practicing physicians and other health professionals. The UPCC Cancer Control Program extends to community hospitals in Pennsylvania and New Jersey. An information letter, Cancer Update, provides UPCC news and cancer education for Center members and other professionals who relate to the UPCC.

277. CHILDHOOD BRAIN TUMOR CONSORTIUM

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A consortium of ten institutions located in North America has been organized, which utilizes the efforts of neuropathologists, pediatric pathologists, a neuroepidemiologist, neurosurgeons, and biostatisticians in a study of childhood brain tumors.

Our principal goal is to obtain accurate prognostic information for tumors at specific sites for the design of therapy for individual children, and for studies of competing therapies. The estimated data base from the ten institutions in the comsortium is between 3,000 and 3,400 children. We will use the first surgical sample for the assessment of the prognostic significance of individual histologic patterns and antecedent clinical features. We will use two classifications, that of the World Health Organization, and another one which uses the biologic characteristics of clustering of histologic patterns and life tables. We will choose which of these classifications provides the most accurate prognostic information for tumors at each site within the brain. This information will be entered into the computer via interactive programs which will provide: 1) specific prognostic information for tumors at each of several sites within the brain for individual patient care decisions by any pathologist or neurosurgeon, and 2) prognostic information for the design of studies of competing therapies.

BIBLIOGRAPHIC REFERENCES: Giles, F., Winston, K., Jasnow, M., Leviton, A., and Hedley-Whyte, E.T.: Densely cellular cerebellar tumors in children. Trans. Am. Neurol. Assoc. 103:265-268, 1978. Gilles, F.: Prognosis in cerebellar tumors. Lab. Invest. 40:4, 1979.

278. CLINICAL CANCER EDUCATION PROGRAM

Reed, M. L., Wayne State University, School of Medicine, Dept. of Oncology, *1014 Rivard, Detroit, Michigan*, 48207, United States of America

Within the last few years Wayne State University School of Medicine expanded from a student class of 120 to 256. A clinical cancer education program for undergraduate medical students is conducted in 9 hospitals and coordinated by a multidisciplinary committee. The curriculum of the first two years is fully integrated and the fourth year is entirely elective. During the third year each clinical department is responsible for transmitting to students the 'core' of its specialty. A Department of Oncology was established to facilitate the coordination of cancer 'core' teaching and to conduct elective senior, graduate, and postgraduate clinical cancer education programs. With support from the Division for Educational Services and Research the faculty is engaged in defining and continually updating the education objectives of cancer teaching at all levels. Educational materials and self-instructional packages are being prepared to assure uniform attainment of these education objectives. Evaluation methods are being developed to test not only the cognitive but also psychomotor and attitudinal aspects of student accom-

plishment. Intensive exposure to oncological problems is provided for house officers in the following disciplines: internal medicine, surgery, otolaryngology, radiotherapy, gynecology, pediatrics, urology, neurosurgery, and orthopedics. A two-year clinical associate program trains ten internists in medical oncology and gives them opportunity to acquire skills in clinical research and education. Subspecialty training programs are also available in gynecological and pediatric oncology.

A continuing clinical cancer education program is being conducted for the practicing physicians of the community.

279. STUDIES OF CHILDHOOD SOLID TUMORS

Simone, J. V., Pratt, C. B., Green, A., Mauer, A. M., Hustu, H. O., Hayes, A., Kumar, M., Beck, W., George, S., Rivera, G., Evans, W., Nakajima, F., St. Jude Childrens Research Hospital, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee*, 38101, United States of America

The overall objective of this project is to develop a coordinated effort to improve the results of treatment of children with solid tumors. This goal is to be achieved through the systematic development of specific treatment regimens for primary care of children with solid tumors as well as for the testing of new agents in Phase I and Phase II studies. In addition, there will be an attempt to develop an epidemiological approach to the children with cancer seen at St. Jude Hospital and also to develop a pharmacological approach to the altered drug pharmacokinetics in children with cancer. The goals set for the current year, the second in this project, are: 1) To develop a multidisciplinary approach to the treatment and evaluation of children with neuroblastoma. 2) To develop a multidisciplinary treatment program for children with osteosarcoma. 3) To plan and initiate an epidemiological program involving the solid tumor patients at St. Jude Hospital with special reference to the unusual occurrence of adenocarcinoma of the colon in childhood and adolescence in this region. 4) To investigate in animal systems and later in children with solid tumors the mechanism by which biotransformation of drugs is altered in experimental neoplasms and the relationship of this effect to polyamines. 5) To study the biological activity of new anticancer chemotherapeutic drugs in children with solid tumors using Phase I and Phase II type testing programs.

280. PEDIATRIC ONCOLOGY PROGRAM PROJECT

Krivit, W., Kersey, J. H., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Pediatrics, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

The Pediatric Oncology Program Project is an integrated grant covering clinical and preclinical studies in oncology and bone marrow transplantation. A major emphasis of this grant is related to therapeutic investigations with emphasis on marrow transplantation for the treatment of acute leukemia, non-Hodgkin's lymphoma, neuroblastoma, aplastic anemia, and for patients at high risk for kidney graft rejection. Children and adults will be treated in these studies. Emphasis is on total lymphoid irradiation and T-cell depletion of donor bone marrow to reduce the risk of development of graft versus host disease. Preclinical investigations form a major portion of this grant, complementing and interdigitating with the therapeutic studies. Included are investigations into the nature and function of committed hematopoietic marrow cells, platelet pathophysiology, cyclophosphamide metabolism, and adrenergic mechanisms in neuroblastoma. Studies of the etiology of childhood cancers will include evaluation of the role of DNA repair mechanisms and tumor viruses.

281. CHILDREN'S CANCER RESEARCH CENTER

D'Angio, G. J., Childrens Hospital of Philadelphia, *3400 Civic Ctr. Blvd., Philadelphia, Pennsylvania*, 19104, United States of America

The program of the Children's Cancer Research Center focuses on the special problems of pediatric oncology and seeks: 1) To devise better and more efficient treatments using clinical and laboratory studies so as to achieve best survival and lowest early and delayed complication rates. 2) To study and understand the late adversities of therapy so that they can be avoided or mitigated. 3) To identify causative factors with a view towards reducing the incidence of childhood cancer.

Integrated clinical, epidemiologic and laboratory investigations will be used to accomplish these objectives. Leukemia, neuroblastoma and other tumors and disease states of neural crest origin will be emphasized in clinical studies and those relating to the genetics, immunology, membrane biology, epidemiology and radiobiology of pediatric neoplasia. The development of minimal therapies for low risk patients and aggressive treatments, including those requiring bone marrow rescue for children at high risk, will be stressed.

The late effects of treatment will be studied, with special reference to the learning disabilities that follow cranial irradiation for leukemia and other cancers.

282. CHILDREN'S CANCER STUDY GROUP

Nesbit, M. E., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Pediatrics, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

The objective of this grant is to facilitate the continued involvement of the University of Minnesota pediatric oncology group in the multi-institutional cooperative therapeutic and non-therapeutic studies conducted by the Children's Cancer Study Group. These studies are conducted using the 'team approach' which involves individuals from a wide range of disciplines. Specific areas of investigation include: design of new therapeutic approaches, assessment of the usefulness of bone marrow transplantation of resistant leukemias, testing of new agents, the treatment and biology of neuroblastoma, drug pharmacology, and the occurrence of iatrogenic effects of treatment in long-term survivors. It is anticipated that the pediatric oncology group at the University of Minnesota will continue to provide significant input into the direction, planning, development and conduct of the clinical investigations and ancillary studies conducted by Children's Cancer Study Group.

BIBLIOGRAPHIC REFERENCES: Shapiro, R., Kim, Y., Michael, A.F., Gerrard, J.M., Nesbit, M., Coccia, P.F., Ramsay, N.K.C., Krivit, W.: L-asparaginase Induced Hypocomplementemia in Acute Lymphocytic Leukemia (ALL) of Childhood. The American Journal of Pediatric Hematology-Oncology. 1:5-7, 1979

283. CHILDREN'S CANCER STUDY GROUP

Feig, S. A., University of California, Los Angeles Campus, School of Medicine, Dept. of Pediatrics, *2025 Zonal Ave., Los Angeles, California*, 90024, United States of America

The multidisciplinary pediatric oncology program at UCLA will participate in the clinical research programs of the Children's Cancer Study Group (CCSG). This will allow UCLA physicians and patients to take part in clinical oncology studies which could not be undertaken in a single institution. UCLA has a program which focuses on certain pediatric neoplasms which have not benefitted from the recent advances in the oncologic armamentarium: a)recurrent acute lymphoblastic leukemia, b) acute myeloblastic leukemia, c) neuroblastoma, and d) histiocytosis-X. UCLA will participate in the development of new investigative programs dealing with the biology, diagnosis, and treatment of these neoplasms with CCSG. These programs will make use of the extensive experience of the UCLA team in the areas of bone marrow transplantation and tumor immunology. In addition, UCLA will initiate local pilot studies relevant to basic and applied pediatric oncology. The data generated by these studies will aid in the development of future limited-institution and Group-wide clinical studies.

284. CANCER CENTER SUPPORT GRANT

Pagano, J. S., Earp, H. S., Grisham, J., Griffith, J., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Medicine, *Manning Dr., Chapel Hill, North Carolina*, 27514, United States of America

The collaborative cancer program at the University of North Carolina at Chapel Hill began in 1969, was funded by a Cancer Center Core Support grant in June, 1975, and was renewed in 1978. The Center began as a basic research center; phased development, starting with build-up of basic research programs in tumor virology, chemical carcinogenesis, tumor immunology, cancer cell biology and drug development followed. Fourteen new faculty have been recruited, and all are funded by the N.C.I., N.S.F., and A.C.S. grants.

In addition to continued growth of the basic science programs, the Center is currently developing its clinical areas as well as a cancer control program which will work through the Area Health Education Centers programs in the state.

BIBLIOGRAPHIC REFERENCES: Biron, C.A., Hutt, L.M., and Pagano, J.S.: Immune Response to Epstein-Barr Virus Induced Cell Surface Alterations. Fed. Proc. 37:1561, 1978. Lemon, S.M., Pagano, J.S., Utsinger, P.D., and Sinkovics, J.G.: Infection of Cultured Hairy Cells with Epstein-Barr Virus: Additional Evidence for a B-lymphocyte Origin. Ann. Int. Med. 90:54, 1979.

285. CLINICAL CANCER RESEARCH PROGRAM

Drezner, M., Seigler, H. F., Huang, A. T., Paulson, D., Metzgar, R., Lyles, K., Laszlo, J., Duke University, School of Medicine, Dept. of Medicine, *Box 3711, Durham, North Carolina,* 27710, United States of America

This program project renewal application seeks support for multidisciplinary clinical research projects dealing with proposals for new methods of diagnosis and treatment of cancer. The leukemia-lymphoma program has projects in autologous bone marrow transplantation, childhood ALL, studies of newly described morphologic particles, research on lymphoproliferative and myeloproliferative disorders, leukemia immunology, platelet antigens, and biochemical pharmacologic studies of new lipid soluble folate antagonists. Additional programs are described in urologic oncology, which include a range of projects from ultrastructure, culture of human cells and clinical chemotherapy through to metabolic studies of new findings of tumor induced osteomalacia, and prognostic factors in prostatic cancer. Interdisciplinary programs in lung cancer, head and neck cancer, breast cancer, malignant melanoma, and brain tumors are also presented in this application.

The central feature of this request is that we have a newly constructed facility in which multidisciplinary research can be conducted and which provides the optimal conditions for education and patient care and enables the collection of research materials for laboratory investigations. The clinical faculty has been greatly expanded and the scope of research increased accordingly.

286. BRAIN TUMOR TASK FORCE, AMERICAN JOINT COMMITTEE FOR CANCER STAGING AND END RESULTS REPORTING

Laws, E. R., Baker, H. L., Vick, N. A., Earle, K., Hendricks, E. B., Kramer, S., Mahaley, M. S., Nesbit, M., Walker, M. D., Wilson, C., Mayo Clinic, Dept. of Neurosurgery, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

The Brain Tumor Task Force of the American Joint Committee is actively engaged in the development of methods of classifying brain tumors. This includes a review of all ongoing chemotherapy protocols, the collection of data collection forms and review of reports on the natural history and therapy of CNS malignancies.

287. ASILOMAR CONFERENCE ON BRAIN TUMOR THERAPY

Levin, V. A., Wilson, C. B., Bigner, D., University of California, San Francisco Campus, School of Medicine, Dept. of Neurosurgery/Neurology & Pharmaceutical Chemistry, *551 Parnassus Ave., San Francisco, California,* 94122, United States of America

The objective of the Asilomar Conference on Brain Tumor Therapy is to foster communication between basic scientists and clinicians engaged in brain tumor therapy.

D. OTHER TREATMENT-RELATED STUDIES

288. THERAPY OF PATIENTS WITH BRAIN TUMORS

Paoletti, P., Universita degli Studi di Pavia, *65 Corso Strada Nuova, 27100 Pavia, Italy*

The Contractor shall exert its best efforts to conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging, and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required, twenty of whom will be newly operated (first craniotomy) patients who will be randomized each year to the Phase III study and fifteen of whom will be patients with recurrent gliomas who will be entered into the Phase II Study.

289. THERAPY OF PATIENTS WITH BRAIN TUMORS

Smith, K. R., St. Louis University, School of Medicine, Dept. of Surgery, *1402 S. Grand Blvd., St. Louis, Missouri,* 63104, United States of America

Independently and not as an agent of the Government, the Contractor shall exert its best efforts to conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent, and clinical stage of the disease, changes in roentgenographic findings,alterations in nuclear imaging and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required. The following protocols will be used to randomize patients to the various studies and to assess toxicity related to various modes of therapy: Protocol for the study of 1,3-bis(2-chloroethyl)-1-Nitrosourea (NSC 409962), 1-(2-chloroethyl)-3-(4-Methylcyclohexyl)-1-Nitrosourea (NSC 95441), and Irradiation in the Treatment of Malignant Glioma of the Brain (Phase III) (BTSG 72-01).

290. THERAPY OF PATIENTS WITH BRAIN TUMORS

Robertson, J. T., University of Tennessee, Center for the Health Sciences, School of Medicine, Dept. of Neurosurgery, *62 S. Dunlap St., Memphis, Tennessee,* 38103, United States of America

The Contractor shall exert its best efforts to conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required.

291. THERAPY OF PATIENTS WITH BRAIN TUMORS

Odom, G. L., Duke University, School of Medicine, Dept. of Surgery, *Box 3711, Durham, North Carolina,* 27710, United States of America

Independently, and not as an agent of the Government, the Contractor shall exert its best efforts to conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging, and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required, twenty of whom will be newly operated (first craniotomy) patients who will be randomized each year to the Phase III study and fifteen of whom will be patients with recurrent gliomas who will be entered into the Phase II study.

292. THERAPY OF PATIENTS WITH BRAIN TUMORS

Alexander, E., Wake Forest University, Bowman Gray School of Medicine, Dept. of Surgery, *300 S. Hawthorne Rd., Winston-Salem, North Carolina,* 27103, United States of America

The Contractor shall exert its best efforts to conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging, and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required, twenty of whom will be newly operated (first craniotomy) patients who will be randomozed each year to the Phase III study and fifteen of whom will be patients with recurrent gliomas who will be entered into the Phase II study.

293. THERAPY OF PATIENTS WITH BRAIN TUMORS

Mealey, J., Indiana University - Purdue University at Indianapolis, School of Medicine, Dept. of Surgery, *1219 W. Michigan St., Indianapolis, Indiana,* 46202, United States of America

Independently, and not as an agent of the Government, the Contractor shall exert its best efforts to conduct studies and

carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging, and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required, twenty of whom will be newly operated (first craniotomy) patients who will be randomized each year to the Phase III study and fifteen of whom will be patients with recurrent gliomas who will be entered into the Phase II study.

294. THERAPY OF PATIENTS WITH BRAIN TUMORS

Young, B., University of Kentucky, School of Medicine, Dept. of Surgery, *Limestone & Euclid, Lexington, Kentucky,* 40506, United States of America

OBJECTIVES: Conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging, and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required, twenty of whom will be newly operated (first craniotomy) patients who will be randomized each year to the Phase III study and fifteen of whom will be patients with recurrent gliomas who will be entered into the Phase II study.

295. THERAPY OF PATIENTS WITH BRAIN TUMORS

Ransohoff, J., New York University, School of Medicine, Dept. of Neurosurgery, *100 Washington Sq. E., New York, New York,* 10003, United States of America

Independently, and not as an agent of the Government, the Contractor shall exert its best efforts to conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging, and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required, twenty of whom will be newly operated (first craniotomy) patients who will be randomized each year to the Phase III study and fifteen of whom will be patients with recurrent gliomas who will be entered into the Phase II study.

296. THERAPY OF PATIENTS WITH BRAIN TUMORS

Hunt, W. E., Ohio State University, Columbus Campus, School of Medicine, Dept. of Surgery, *370 W. 9th Ave., Columbus, Ohio,* 43212, United States of America

Independently, and not as an agent of the Government, the Contractor shall exert its best efforts to conduct studies and carry out clinically indicated modes of therapy as these relate to patients with malignant gliomas and other types of intracranial tumors, and to determine the efficacy of a number of therapeutic approaches, such as possible intrarelationships to morphologic type, extent and clinical stage of the disease, changes in roentgenographic findings, alterations in nuclear imaging, and pharmacotoxicology of selected agents in certain cases. A minimum number of 35 patients is required, twenty of whom will be newly operated (first craniotomy) patients who will be randomized each year to the Phase III study and fifteen of whom will be patients with recurrent gliomas who will be entered into the Phase II study.

297. PROCESSING OF CLINICAL PATIENT RESEARCH DATA

Schmitt, D. H., Leiby, N. J., Control Data Corp., *6003 Executive Blvd., Rockville, Maryland,* 20852, United States of America

The scope of the project is to: 1) Code, keypunch, verify/validate, edit/update, store and retrieve the data from the medical records of patients with malignant gliomas entered into the BTSG study protocols No. 75-01, No. 76-20, No. 77-01, No. 77-02, and No. 78-20 on a continuing basis. 2) Analyze the data derived from all BTSG protocols and provide the analysis to the BTSG Central Office in the form of master data listings, interval listings, comparability listings, Wilcoxon analysis, chi-square testing and other statistical procedures that may be applicable. Approximately six (6) data updatings are anticipated yearly. 3) Provide support on an ad hoc basis to develop the computer programs, plotting and reporting capabilities necessary to furnish listings and statistical analysis of selected data parameters which would include, but not be limited to: Karnofsky ratings, neurological examination results, comparability data, Wilcoxon median computations, prognostic factors, and intra-protocol data comparisons as requested by the BTSG Central Office. A maximum of approximately ten (10) such ad hoc requests are anticipated yearly. 4) Provide consultant services in data management problems as required.

298. CONSULTATION ON CLINICAL TRIALS

Byar, D. P., Gail, M. H., Green, S. B., Muenz, L. R., Corle, D. K., Levin, D. L., Rubinstein, L. V., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Clinical & Diagnostic Trials Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The purpose of this project is to provide consultative services in statistical and epidemiological methodology in the design, interpretation, and evaluation of clinical trials and other studies requiring this kind of expertise. For some trials the section provides full statistical support including development of detailed study plans, randomization of patients, collection, processing, editing, and analysis of data, and preparation of scientific papers. At present, members of the Section are involved in studies concerned with prostate cancer, testis cancer, breast cancer, lung cancer, brain tumors, and mycosis fungoides. In addition to these major projects, a variety of other consultative activities, including review of scientific articles, arise in response to special requests.

BIBLIOGRAPHIC REFERENCES: Byar, D.P.: Review of the Veterans Administration Studies of Cancer of the Prostate and New Results Concerning Treatment of Stage I and II tumors. In Pavone-Macaluso, M., Smith P.H., and Edsmyr, F. (Eds.): Bladder Tumors and Other Topics in Urological Oncology. New York, Plenum Press, 1980, pp. 471-492. Byar, D.P.: VACURG studies of post-prostatectomy survival. Scand. J. Urol. and Nephrology, May, 1980.

299. RTOG STATISTICAL COORDINATING CENTER

Hanley, J. A., Gelber, R., Zelen, M., Mehta, C., Pagano, M., Schoenfeld, D., Stanley, K., Neff, R., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts,* 02115, United States of America

The activities of the past year included preparation of statistical analyses dealing with primary brain tumors (1), metastatic brain tumors (4), head and neck tumors (6), esophageal cancers (1), lung cancer (2), pelvic tumors (1), osseous metastases (1), and hepatic metastases (1). In addition, several manuscripts were authored on statistical methodology pertinent to clinical trials.

300. CHANGING EFFECTS OF HEMISPHERECTOMY

Smith, A., Campbell, A., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Physical Medicine & Rehabilitation/Education, *1335 E. Catherine St., Ann Arbor, Michigan,* 48104, United States of America

The proposed work will include neuropsychological examination of new patients and systematic follow-up studies of 1) previously tested patients with hemispherectomy for intractable seizures (in infantile hemiplegics) and for brain tumor; 2) commissurotomy; and 3) subtotal agenesis of either hemisphere.

Comparisons of preoperative and/or initial and later postoperative studies of these patients provide data indicating principles underlying the organization, disorganization and reorganization of cerebral functions following pre-, and post-natal insults at different stages in the development of the brain including: 1) The nature, rate and extent of 'functional plasticity' of the brain with advancing age, 2) the extent to which each hemisphere participates and specializes in certain language and nonlanguage functions as the brain matures, 3) limitations and principles underlying the reorganization of cerebral functions following hemispherectomy, commissurotomy or subtotal agenesis of either hemisphere.

BIBLIOGRAPHIC REFERENCES: Smith, A. Practices and Principles of Clinical Neuropsychology. Intern. J. Neuroscience, 1979, 9:233-238. Smith, A. Principles underlying human brain functions in neuropsychological sequelae of different neuropathological processes. In S.B. Filskov and T.J. Boll (Eds.), Handbook of Clinical Neuropsychology. New York: Wiley Interscience, 1980 (in press).

301. TREATMENT OF NEUROBLASTOMA

Hutchinson, W. B., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

This is part of a broader project. A summary of this subproject is not available.

302. CANCER THERAPY - CENTRAL NERVOUS SYSTEM NEOPLASMS (HUMAN)

Higby, D. J., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

303. PROTOCOL FOR MALIGNANT GLIOMA OF THE BRAIN

Mahaley, M. S., Duke University, School of Medicine, Dept. of Medicine, *Box 3711, Durham, North Carolina*, 27710, United States of America

This is part of a broader project. A summary of this subproject is not available.

304. THERAPY OF PATIENTS WITH BRAIN TUMORS

Hunt, W. E., Ohio State University, Columbus Campus, School of Medicine, Dept. of Medicine, *370 W. 9th Ave., Columbus, Ohio*, 43212, United States of America

This is part of a broader project. A summary of this subproject is not available.

305. TIME-DOSE STUDIES IN CENTRAL NERVOUS SYSTEM METASTASES (HUMAN)

Poulter, C., University of Rochester, School of Medicine & Dentistry, Dept. of Surgery/Radiology, *601 Elmwood Ave., Rochester, New York*, 14627, United States of America

This is part of a broader project. A summary of this subproject is not available.

306. NEURO-ENDOCRINE FUNCTION IN CHILDREN AFTER LONG-TERM MALIGNANCY THERAPY

Fisher, J. N., University of Tennessee, Center for the Health Sciences, School of Medicine, Dept. of Medicine, *62 S. Dunlap St., Memphis, Tennessee*, 38103, United States of America

This is part of a broader project. A summary of this subproject is not available.

307. BRAIN TUMOR UNIT

Bigner, D. D., Duke University, School of Medicine, Dept. of Pathology/Surgery, *Box 3711, Durham, North Carolina*, 27710, United States of America

This is part of a broader project. A summary of this subproject is not available.

V. IMMUNOLOGICAL ASPECTS OF NERVOUS SYSTEM TUMORS

A. TRANSFORMED AND NORMAL CNS CELL SURFACE ANTIGENS

308. STUDY OF SURFACE ANTIGENIC CHARACTERISTICS OF HUMAN CULTURED GLIOBLASTOMA CELLS

de Tribolet, F. N., Zander, E., Schnegg, J. F., Carrel, S., University Hospital Center for the State of Vaud, Service de Neurochirurgie, *CH1011 Lausanne, Switzerland*

OBJECTIVE: To investigate surface antigenic properties of cultured human glioblastoma cells.

APPROACH: Membrane fractions of 2 established human glioblastoma cell lines are prepared by nitrogen cavitation. Antisera against each of the glioblastoma cell membrane preparations are raised in rabbits. The antisera are then extensively absorbed to remove species-specific and HLA activities. Subsequent absorptions are performed with unrelated tumor cells and finally with normal human brain. The specificity and cross-reactivity of the absorbed heteroantisera are tested with the complement-dependent and the antibody-dependent cell-mediated cytotoxicity assays. 51Cr-labelled cells from 8 different human glioblastoma cell lines are used as targets. The heteroantisera will be further characterized by immunoprecipitation analysis.

In a parallel study BALB/c mice are immunized with whole human glioblastoma cells, and monoclonal antibodies are produced by hybridomas.

PROGRESS: Using the monoclonal antibodies three types of antigens have been identified: two of them are present on human malignant gliomas exclusively, but the third one is present on neuroblastoma, melanoma and normal adult and fetal brain as well as on malignant glioma cells.

309. TUMOR AND DIFFERENTIATION ANTIGENS IN THE IMMUNE CONTROL OF NEUROBLASTOMA

Revoltella, R. P., Bertolini, L., Butler, R. H., Businaro, R., Bosman, C., Torre, A., Vigenti, E., Ghelardini, P., Brahe, C., Consiglio Nazionale delle Ricerche, Lab. of Cell Biology, *Rome, Italy*

OBJECTIVE: To isolate and characterize structural antigens responsible for the specific anti-tumor immune response in neuroblastoma (NB) tumor-bearing A/J mice.

APPROACH: 1) Isolation of clones and somatic cell hybrids of NB which are high-, low-, or non-producing for specific alloantigenic structures; 2) isolation and immunochemical characterization using monoclonal antibodies of tissue-type and tumor-associated antigens from cloned NB cells; 3) protection of syngeneic mice against NB cell challenge by inoculation of individual NB-associated antigens.

PROGRESS: Various clones and hybrids of the C1300 NB cell line which differ in particular structures antigenic in a syngeneic system have been isolated. In addition, a new line of neuroblastoma, NB-SV, has recently been obtained by infecting embryonic sympathetic neurons from strain A mice with SV40 virus. Antigens have been solubilized from cloned NB cells and partially purified. These fractions retained antigenicity as defined by a variety of immunological tests, including radioimmunological methods using antibodies from syngeneic mice immunized against fixed NB cells or NB cell membranes. Monoclonal antibodies anti-NB have been obtained by somatic cell fusion between plasmacytoma cells, P3x63Ag8, and spleen lymphocytes from A/J mice a) NB tumor-bearing, b) immunized against NB cell membranes. The specificity of these antibodies is being investigated using purified C-type RNA-viral antigens and the solubilized fractions from NB cells.

310. HUMAN DIFFERENTIATION ANTIGENS AND SOMATIC CELL HYBRIDS

Beverley, P. C., Wallace, D., Evans, A., Linch, D., Janossy, G., Bradstock, K., Imperial Cancer Research Fund, Tumor Immunology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

We have continued to produce monoclonal antisera to human cell surface antigens by the technique of somatic cell hybridization. An antiserum (anti-HLe-1) recognizing a common leukocyte antigen has been most extensively analyzed. Only cells of the lymphoid, myeloid and erythroid series bear the antigen, but while mature lymphocytes, monocytes and granulocytes retain it, it is lost during maturation of erythrocytes and platelets. Fractionation of bone marrow by fluorescence-activated cell sorting reveals that CFU-C are weakly positive. Further enrichment of myeloid precursor cells by removal of mature myeloid cells from the weakly stained population should be possible by density gradient fractionation. The antibody also shows promise as a diagnostic reagent, particularly in distinguishing lymphoid from non-lymphoid tumors in tissue sections.

A number of other surface markers, including two that are restricted to mature granulocytes, are under investigation. We have detected, though not yet cloned, antibodies which distinguish brain tumor-derived cell lines from other cell types.

We have also made human T x human T cell hybrids using azaguanine-resistant T-ALL-derived lines as the immortal partner. The properties of these lines are under investigation.

311. ANTISERA TO HUMAN NEUROBLASTOMA CELLS

Kemshead, J., Bicknell, D., Greaves, M., Imperial Cancer Research Fund, Membrane Immunology Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

We are attempting to produce antisera that will unequivocally identify small numbers of neuroblastoma cells infiltrating bone marrow of patients.

Studies on antisera raised to several neuroblastoma cell lines, either in rabbits or by somatic cell hybridization studies, suggest a marked heterogeneity of cell surface antigens on different lines. Examination of the binding of neuro-toxins (bungarotoxin, tetanus) and cholera toxin also suggests substantial variation in the expression of surface binding sites for these ligands. These observations have further been confirmed by study of bone marrow samples, known by histological examination to be heavily infiltrated with neuroblasts. Whilst with certain antisera some samples showed good reactivity, with others no binding could be demonstrated.

The marked heterogeneity of surface antigenic expression within the tumors defined as 'neuroblastoma' may be explained by the observation that neuroblastoma is capable of spontaneous or induced differentiation/maturation to a benign tumor (ganglioneuroma). Different antigenic expression may correspond to the arrest of cells at different maturation stages. In vitro studies on the differentiation of human neuroblastoma lines are in progress to test this possibility.

Hybridoma (monoclonal) antibody-defined surface antigens so far tested are shared with at least one hemopoietic lineage (e.g., granulocytes or B cells). Differentiation-linked cell surface antigens on hemopoietic cells, T cell antigens, HLA-D/'la' antigens are not detectable on neuroblastoma cells.

312. IMMUNOCHEMICAL CHARACTERIZATION OF NEURONAL CELL SURFACE ANTIGENS

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The nerve cell surface presumably plays a critical role in nerve function and in nerve cell-cell recognition and may therefore reflect the pathways of differentiation of distinct nerve cell types. Schachner and co-workers have prepared a series of distinct antisera that specificially detect antigens unique to the cell surface of murine nervous tissue. I propose to biochemically characterize and isolate antigens detected by two of these antisera, designated NS-1 and NS-3. Tissue cultured cell lines bearing NS-1 or NS-3 antigens will be radiolabeled by cell surface labeling techniques specific for protein or carbohydrate and NS-1 or NS-3 antigens will be isolated from detergent solubilized cell fractions using NS-1 or NS-3 antisera. Antigens isolated by electrophoresis or thin layer chromatography will be used to generate more specific antisera that will facilitate biochemical identification and immunological fractionation of differentiated nerve cell types. These more specific antisera will also be useful in analyzing nerve cell-cell interactions in living nerve cells.

313. IN VITRO IMMUNIZATION WITH AUTOCHTHONOUS HUMAN TUMOR

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OBJECTIVE: Demonstrate the existence of specific human tumor-associated antigens and show in vitro increased immune responses to such antigens after in vitro immunization with autochthonous tumor. Approximately 60 tumor specimens per year from patients with carcinoma of the lung, astrocytomas, and osteo- and soft tissue sarcomas will be studied. Attempts will be made to characterize the nature of the effector cell. Any modification of the study design must have the prior approval of the Project Officer before being instituted.

314. SURFACE ANTIGENS OF NEURAL CELLS

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Through the use of specific antisera we will study (1) the surface antigens found on cloned lines of cells from rat and mouse brain and (2) the expression of these same antigens in normal nervous tissue. These investigations will be aimed specifically at correlating cell surface markers with cell function and at mapping specific cell populations during the development of the mammalian nervous system.

The cloned cell lines used in these studies have been identified as neuronal or glial on the basis of electrophysiology and a series of ion flux assays which can detect receptors for neurotransmitters and voltage-dependent Na plus, K plus, and Ca plus plus channels. Six antigens (N1-N6) have been identified which are specific for cell lines classified as neuronal, and four others (G1-G4) have been shown to be specific for cell lines classified as glial. We will continue to enlarge our library of antigens by preparing new antisera against cell lines that are not well characterized by the existing antigens. We will also attempt to prepare more monospecific antisera by immunization with purified antigens and by making hybridomas to produce monoclonal antibodies.

Most of the antigens defined by these studies have been located in normal brain in a rough way by use of quantitative immunoabsorption. In addition, more detailed mapping of cells has been done in the case of G4, a high molecular weight glycoprotein which is present on a distinct population of cells in sections and primary cultures of cerebellum. Future studies will emphasize mapping of various antigens as a function of development - i.e., the sequence of appearance of antigens, the origin and fate of cells bearing the antigens, and the types of cells expressing the antigens.

315. IMMUNOLOGICAL ANALYSIS OF THE SURFACE ANTIGENS OF HUMAN GLIAL BRAIN TUMOR CELLS

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We have extensively characterized a large panel of established human tumor cell lines of gliomatous and non-central nervous system origin by the use of polyspecific heteroantisera raised in nonhuman primates. This analysis led to the demonstration of normal adult and fetal human brain-associated antigens, cross-reactive glioma antigens, and potential idiotypic glioma antigens on the cell membranes of cultured human glioma cells. In order to produce antibodies of greater specificity, we have developed the hybridoma monoclonal antibody technique and are producing anti-human glioma hybridoma antibodies. Several hybridomas have been rapidly screened by a cell surface radioimmunoassay and 14C-nicotinamide release assay; reactivity patterns of various degrees of specificity have been detected, including hybridomas which react with all human cell cultures and those which react primarily with cells of neuroectodermal origin. In addition, we are analyzing the anti-glioma activity of human antisera resulting from the immunization of glioma patients with allogeneic glioma cells.

We propose to produce several anti-human glioma hybridomas by immunization with cultured glioma cells and tissue biopsy samples and to define the specificities detected as adult or fetal organ specific, cross-reactive neuroectodermal, glioma associated, or potentially idiotypic in origin, following the elimination of species-specific reactivity by adsorption with multiple lymphoid cell populations if necessary, and demonstration of the lack of HLA reactivity, readily induced by glioma cell immunization. We will then determine both cell surface and tissue distributions of the glioma-associated specificities in cell-surface radioimmunoassay, and 14C-nicotinamide release, indirect immunofluorescence, and peroxidase-labelling assays of dispersed cells and tissue sections with monoclonal antibodies and human antiglioma sera. The antibodies of defined specificity will then be used to monitor the isolation, preparation, and characterization of cell-free glioma-associated antigenic specificities.

316. CELL SURFACE ANTIGENS OF GLIAL BRAIN TUMOR CELLS

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Surface antigens of human glial brain tumor cells and VM murine spontaneous anaplastic astrocytoma cells were investigated by binding (indirect membrane immunofluorescence) and complement-dependent cytotoxic antibody (14C-nicotinamide release and dye-exclusion) assays. Antisera from nonhuman primates immunized with glioblastoma multiforme tissue or cells

from permanent human glial brain tumor cell lines have been extensively absorbed to analyze which human glioma cell surface antigens may be species-, tissue-, or glial tumor-specific; oncofetal; or cross-reactive with non-glial tumor cell antigens. We have established an inbred colony of VM/dk mice, an in vivo transplantable VM astrocytoma line, and 3 VM astrocytoma cell lines. We will extend the initial finding of human glial tumor-associated antigenicity with additional primate antisera now in production and an expanded panel of neoplastic and non-neoplastic adult and fetal glial and non-glial human cell lines. Syn-, allo-, and xeno-geneic antisera raised against the VM astrocytoma will be analyzed for activity against murine oncornaviruses; antisera suitably absorbed will be analyzed for astrocytoma-associated antibody activity. In both systems we will extract from culture supernatants or cell membrane preparations antigenically active moieties by monitoring the process in inhibition assays with absorbed specific antisera. Radioimmunoprecipitation of labeled reactive antigenic moieties will be followed by polyacrylamide gel electrophoresis. Partial purification of solubilized antigens will be performed by gel filtration, ion exchange chromatography, and other gentle methods of protein separation. Specific antisera and solubilized antigens will be used to determine if body fluids of patients or cancerous mice contain glioma-distinctive antigens, measurement of which may serve diagnostic, disease process-monitoring, or immunotherapeutic purposes.

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317. HUMAN NEUROBLASTOMA CELL SURFACE ANTIGENS

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There is a well recognized need to improve the therapy of childhood neuroblastoma. Our studies aim to characterize neuroblastoma cell surface antigens (CSA) by serologic and biochemical criteria and then to develop immunologic tests and immunotherapy utilizing them. To characterize CSA, we use cultured neuroblastoma cells and non-cultured tumors and tissues, monoclonal antibodies produced by lymphocyte hybridomas, rabbit and human antisera, the radioiodinated protein A assay for cell surface bound antibodies, and isoelectric focusing/polyacrylamide gel electrophoretic analysis of immunoprecipitated radiolabeled CSA. Emphasis will be placed upon identifying CSA which are specific for neuroblastoma cells or for fetal cells. Immunologic tests will include quantitation released (shed) CSA and determination of CSA phenotype. These tests may provide means of classifying tumors and of predicting and monitoring the response of neuroblastoma to therapy. Some CSA may serve as targets for immunologically specific cytotoxic or cytostatic reactions providing considerable therapeutic potential. Clinical studies of children with neuroblastoma and others will determine the incidence of antibody responses to CSA as an indicator of immunogenicity, and in vitro and in vivo model studies will determine the ability of antibodies to affect neuroblastoma cell survival and growth. Development of immunologic tests and immunotherapy should significantly contribute to our long-term aim of achieving optimal therapy for children with neuroblastoma.

318. CHILDHOOD NEUROBLASTOMA - ANTIGENIC CHARACTERISTICS

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The prognosis of neuroblastoma, one of the most common childhood cancers, has not improved over the last two decades. It is almost universally fatal in older children with disseminated disease. In contrast, neuroblastoma of infants younger than one year may spontaneously remit, or it is frequently controlled and cured with current therapy. The long-term goal of this proposal is to critically define these factors and to determine their role in the clinical characteristics and therapeutic response of childhood neuroblastoma. Specifically we will determine if lymphocyte or serum mediated cytotoxicity against neuroblasts is due to normal differentiation antigens or to tumor specific antigens, compare the cytotoxicity of bone marrow, lymph node and blood lymphocytes against autologous or allogeneic neuroblasts, and establish if alpha-fetoprotein, detected on established neuroblastoma cell lines, is also expressed on viable neuroblasts from patients and investigate the effect of neuroblast-associated alpha-fetoprotein on host immune response. Systematic studies of these parameters should establish the biological differences between neuroblastoma of younger and older children and provide the knowledge required for the development of effective therapy.

319. IMMUNOREGULATION BY SHED TUMOR MEMBRANE COMPLEXES

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Cancerous cells develop increasing lethal effects on their host as growth proceeds from early phases to later acute or metastatic phases. It has been proposed that by shedding cell membrane antigens and complexes tumor cells develop the capacity to modulate and eventually suppress the host's immune responses. This project will focus on attempting to understand the biological, immunological, biochemical, and biophysical properties of shed-suppressive membrane complexes and the changes that take place as tumor cells spread from their initial primary clones to penetrate other sites. Discrete cell populations will be fluorochrome labeled and preparatively sorted to permit defined conditions for analysis. The membrane changes will be compared to previous ongoing work on membrane shedding from leukemia, lymphoma, and neuroblastoma cells. Thy-1 antigen (glycolipid and glycoprotein components of normal T cells and the cancer cells to be used in this study) has been shown to be a unique antigenic and functional component of the shed material and will serve as one of the main centers of attention in the proposed investigation. In addition to cultured lymphoma and neuroblastoma cells, primary cancer cells from murine hosts in various states of neoplastic development will be placed into culture systems for these studies. Also serum from tumor bearing animals will be studied for the presence and state of shed antigens and complexes. Various developing and functional classes of lymphocytes will be assayed as targets of shed materials. Precursor and primary B cells and their interactions with T helper, suppressor, effector, and amplifier systems will be the focus of these studies. Biochemical and biophysical properties of membrane complexes and molecular components of the various forms and stages of neoplastic cells and their subsequent targets in the immune system will provide information for devising a means of interfering with this suppressor mechanism of cancer cells and restoring the host immune system to full potential for subsequent destruction of the tumor.

320. BRAIN-SPECIFIC ANTIGENS - GENETICS AND BIOCHEMISTRY

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This research is based on the hypothesis that cell-surface molecules play critical roles in the differentiation and regulation of complex systems such as the nervous system. To explore this hypothesis, differentiation and tumor-specific antigens of the mouse nervous system will be recognized and defined using monoclonal antibodies from hybridomas. Cell populations defined by their antigenic differences will be purified and used in genetic, biochemical and biological studies of the component cells of the nervous system and of their surface molecules.

321. IMMUNITY TO HUMAN CANCER - FUNCTIONAL COMPONENTS

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Studies are being conducted in three areas: 1) isolation of human glioma-associated cell surface antigens; 2) characteriza-

tion of a substance, found in the serum of humans and mice that bear anaplastic tumors, that inhibits fundamental cell-cell interactions; 3) study of the capability of normal cells, unrelated to the immune system, which have the capacity to inhibit tumor cell replication and biological activity. These studies employ mixed aggregates of normal and neoplastic human cells.

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322. BRAIN ASSOCIATED HEMATOPOIETIC ANTIGENS

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The presence of brain associated hematopoietic antigen on cultured bone marrow cells (Dexter cultures) is being examined. Brain associated stem cell antigen and the brain associated antigen found on pre-T cells are being determined by treating the cultured cells with appropriately absorbed rabbit anti-mouse brain antiserum and testing their ability to generate CFU-S or Thy-1 positive cells in response to thymopoietin. Bone marrow cells are being cultured in the presence of thymic epithelium cells and the expression of brain associated theta antigen is being determined.

323. POLYMORPHIC NEURONAL SURFACE PROTEINS

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In this project, I will ask whether there are neuronal cell surface molecules that share a common protein backbone, but display structural variations from neuron to neuron. Such molecules may well be involved in defining neuronal specificity. My approach will be to use monoclonal antibodies as monospecific reagents, and different human neuroblastoma-derived cell lines as sources of homogeneous material. The basic sequence of experiments will be: (1) To develop a panel of monospecific antibodies, each of which recognizes several different neuroblastoma-derived lines as well as normal nervous tissue, (2) to use each antibody to immunoprecipitate its antigen from the surface of two or more of the cell lines, (3) to use SDS-PAGE, IEF and peptide map analyses to ask whether any of the molecules show structural microheterogeneity from cell line to cell line.

In carrying out this project, I will of necessity be characterizing those molecules that are present on human neuroblastoma-derived cell lines and are also shared by normal nervous tissue. Thus, I will be starting to form an immunological and biochemical map of the normal human neuron.

324. TERATOCARCINOMA AND EMBRYONAL TUMORS - SURFACE ANTIGENS

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This application proposes to follow serologically embryonic surface antigens in the mouse that are characteristic of specific early stages of cell differentiation both in the embryo and on embryonal tumor cells, specifically teratocarcinoma and a genetically caused medulloblastoma-neuroblastoma. The project will make use of a series of mutant genes at the T-locus which are known to: 1) Interfere with differentiation at specific times in development, 2) specify cell surface antigens on sperm and in the embryo, and 3) in the one case studied, cause an embryonal tumor. We would like to know the time course of the expression of these antigens, if they are functional, and what role the mutant form of the antigen plays in malignant differentiation. These objectives involve transplantation of mutant embryos, obtaining transplantable tumors from t-mutant cells, and serological definition of embryonic surface antigens by means of cytotoxicity tests and absorption studies.

325. PRESENCE AND FUNCTION OF IA POSITIVE CELLS IN THE BRAIN

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The primary objective of this proposal is to study the immune system of the central nervous system. We will focus on the characterization of Ia-bearing cells in terms of morphology, surface markers and function.

The identification and characterization of Ia positive cells are critical since Ia-positive cells of the periphery are involved in a complex array of immune functions. Perhaps most importantly, they appear to be primary antigen-presenting cells. The localization of these Ia-bearing cells in the brain has been accomplished by immunoperoxidase techniques. The further characterization of these cells can be accomplished by morphologic studies and by the identification of specific cell-surface markers. Finally, these cells will be characterized by their ability to function in in vitro immune systems. These assays will be performed on Ia positive cell fractions prepared by density gradient separation and anti-Ia antibody-coated polystyrene dishes.

326. IMMUNOCYTOCHEMISTRY OF HUMAN BRAIN TUMORS

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We have screened over 150 human brain tumors for the presence or absence of SV40 T-antigen. Two tumors were highly suspicious for the presence of T-antigen: one a human ependymoblastoma, the other a choroid plexus papilloma. In addition, we have developed a radioimmunoassay for T protein, which is applicable to extracts of human tumors. In addition, our immunocytochemical studies have been extended to include the identification of various hormones and pituitary tumors. This includes growth hormone, ACTH, and prolactin. We have developed a frozen section method, which will be applicable to the study of human pituitary tumors removed at surgery. We have concluded that T-antigen is not a significant component of human tumors, particularly meningiomas, but that a frozen section technique may have application in surgical treatment of pituitary adenomas.

327. NERVE AND MUSCLE SURFACE MACROMOLECULE INTERACTION

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The overall objective of this project is to determine the molecular basis for the interaction of nerve and muscle. We have previously characterized the Band 1 protein, a murine nervous system specific cell surface antigen. Currently, efforts to determine whether Band 1 is a marker for a particular subclass of neurons center around analysis of activity in the cerebella of mice with characterized nervous system mutations. New antisera to Band 1 are being developed for histologic analysis of neural tissues. The search for additional neuron-specific cell surface components is continuing with the analysis of chick brain primary cultures which are more than 95% neuronal. Cell surface components are identified by lactoperoxidase iodination and PDP/NaB3H4 labelling. Analagous cultures from rat or mouse brain currently contain unacceptably high levels of (putative) glial contamination which must be eliminated prior to cross-species comparisons of neuron cell surface components. Spinal cord cultures are being prepared in the same fashion for analysis of a population enriched in cholinergic cells.

328. THE SYNTHESIS AND INTERACTION OF COMPONENTS MEDIATING SPECIFIC EMBRYONIC CELL ADHESION

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The mechanisms which mediate the formation and stability of intercellular attachments are critical to understanding both normal and aberrant developmental phenomena. The relative affinity of one cell for another is presumed to change during development so that cells form a continuum of differing architectural patterns with their neighbors, culminating in morphology characteristic for a particular organ or tissue. In addition

changes in the adhesive capacity of neoplastic cells may be related to their ability to metastasize and invade surrounding tissue. Characterization of the surface structures involved in the formation of adhesions, their synthesis and turnover in the embryonic chick neural retina are the objectives of this research.

329. MECHANISMS OF ADHESION AMONG MURINE CELLS

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An immunological protocol for the study of cell adhesion, based on antibodies that can inhibit cell adhesion and the identification of cell surface antigens that bind to these antibodies, has been used to isolate a cell adhesive molecule from chick embryonic neural retina. The proposed investigation applies this protocol to a study of adhesion using murine cell lines, including neuroblastoma and embryonal cell carcinoma, at various stages of differentiation. Genetic aberrations affecting development, including T-locus mutations, trisomies, and nervous system mutations such as 'reeler', will also be examined. This study is aimed at the isolation of molecules active in cell adhesion at various times of development, and should aid in understanding normal and disturbed embryogenesis as well as certain aspects of malignancy.

330. ETHYLNITROSOUREA-INDUCED RAT GLIOMAS

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This research entails an analysis of the immunology of early-generation transplantable derivatives of ethylnitrosourea-induced F-344 rat malignant gliomas. More specifically, by means of in vivo tumor rejection assays and Winn tumor neutralization tests, the aims are (a) to determine if these gliomas express individually unique or cross-reactive tumor-specific transplantation antigens, (b) to measure the extent to which the afferent and efferent limbs of cellular immunity are active against these tumors when they are grafted in the brain, (c) to explore whether BCG-priming/PPD-coupling are effective methods of augmenting immunologically mediated rejection of glioma transplants in either subcutaneous or intracerebral sites, and (d) to clarify the role of thymus-derived cells in mediating either tumoricidal anti-glioma immunity or tumor growth-enhancing processes.

331. ANTIGEN MARKERS IN CHARACTERIZING AND SEPARATING NERVE CELLS (MAMMALS)

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This is part of a broader project. A summary of this subproject is not available.

332. CELL-SURFACE ANTIGENS OF RENAL CANCER, ASTROCYTOMA, LEUKEMIA AND LYMPHOMA

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This is part of a broader project. A summary of this subproject is not available.

B. HOST HUMORAL AND CELLULAR IMMUNITY TO CNS TUMORS

333. STUDY OF THE IMMUNE RESPONSE INDUCED IN THE HOST BY PRIMARY HUMAN BRAIN TUMORS

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DESCRIPTION: The objective of the research program is first the demonstration, then the quantitation of the immune response mounted by the brain tumor patient against his tumor (only glioma patients are being studied now). As a general approach we looked for: 1) circulating antibodies and 2) sensitized lymphocytes in the peripheral blood, which can react specifically with allogeneic tumor cells maintained in tissue cultures (short-term and long-term). Existence of a humoral response was demonstrated by membrane immunofluorescence and suggested the presence of two glioma-associated antigens on the surface of cultured cells: the first one (A) being shared by benign and malignant tumors, and the second (G) restricted to the malignant ones. The presence of memory T-lymphocytes was also demonstrated with a two-step chromium-51 release assay, which proves now to be a helpful in vitro diagnostic assay in clinical practice. Further research involves examining our patients for antibody-dependent cell-mediated cytotoxicity (ADCC). Attempts at characterization of the A and G antigens by salt extraction, solubilization and biochemical study are under way.

A further question is whether these two glioma-associated antigens, which were serologically defined, are also responsible for the cellular immunity. Primary immunization of normal lymphocytes against glioma-associated antigens with the mixed lymphocyte-tumor culture gives promising results in this perspective.

Parallel to this research, continuous work is performed on the characterization of our cultured glioma cell lines (morphology, growth pattern, presence of glia-specific proteins, invasiveness in vitro, immune markers).

334. EVALUATION OF HUMORAL RESPONSE OF GLIOMA PATIENTS TO POSSIBLE TUMOR-ASSOCIATED ANTIGENS USING ADDC ASSAY (ABBREV)

de Tribolet, F. N., Zander, E. F., University Hospital Center for the State of Vaud, Service de Neurochirurgie, *CH1011 Lausanne, Switzerland*

OBJECTIVE: To study the humoral response of glioma patients to a possible common surface tumor antigen(s) using the antibody-dependent cell-mediated cytotoxicity (ADCC) assay.

APPROACH: Sera from patients with glial tumors were collected at several intervals in the course of the disease and tested in vitro using an ADCC assay. Cells from 8 long-term cultures of human glioblastoma were used as targets in a 6-hour 51Cr release assay using peripheral blood leukocytes from normal individuals as a source of effector cells. The same tests were repeated using rabbit complement.

PROGRESS: Sera from 59 patients were tested against cells from 8 glioblastoma lines. Ten patients were found to have cytotoxic antibodies against targets from one or more cell lines as compared to 5 out of 25 normal blood donors. Extensive cross-reactions between glioma cells were found. However, the cross-reactivity was incomplete and the pattern of reactivity different for each serum tested.

The positive sera were tested against 5 tumor cell lines of unrelated origin and found to have a similar cytotoxic activity as compared to that obtained with glioblastoma cells.

335. IMMUNOLOGICAL MONITORING OF PATIENTS BEARING CEREBRAL ANAPLASTIC GLIOMAS

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In order to study the pathology of malignant primary cerebral tumors, a collaboration has been established between the Department of Neurosurgery of the University of Rome School of Medicine and the Departments of Microbiology and General Pathology of the same University. To this end, in the second half of the 1979 we started isolating some cellular populations from biopsies of patients bearing glial tumors. The procedure was as follows: After having been dissociated mechanically or by using trypsin, in a RPMI medium (or F 100) supplemented with 15% of FCS mycoplasma free and 1% of penicillin-streptomycin, biopsies were incubated in a humidified atmosphere of 5% CO2 at 37 degrees C. The cellular populations we obtained were characterized by (1) in vitro cellular behavior (by the brain invasion test of baby rat), (2) presence of surface glia-specific antigens (GFA, S100, albumin), (3) biochemical properties (sensitivity to ouabain, c-AMP 3'-phosphohydrolase activity). Moreover, either before or after surgery, we took blood samples from the patients to be able to evaluate their actual specific immune response (humoral and cellular: indirect immunofluorescence test, PAP test, in vitro lymphocyte blastitation test). At present we have 8 tumoral in vitro populations (5 glioblastomas and 3 anaplastic astrocytomas) and 18 sera (12 from patients harboring glioblastomas and 6 from 'unaffected' controls). IMF and PAP tests have shown a specific humoral response. Also in some cases we observed a blastic response of

patients' lymphocytes in mixed unidirectional cultures of gliomatous cells, fixed with glutaraldehyde.

336. NATURAL AND TISSUE-SPECIFIC IMMUNITY TO HUMAN NEOPLASMS

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Methods have been developed for enrichment and characterization for human natural killer (NK) cells. The morphology, functional characteristics and certain surface characteristics of human NK cells have been described.

The effect of interferon on the fractionated human NK cells has been studied. We have demonstrated that NK cells produce interferon when contacted with tumor target cells and the produced interferon recruits an initially non-cytoxic 'pre-NK' cell population to killer cells with the properties of NK cells. There is thus, within the NK system itself a self-amplifying positive feed-back mechanism which may be potentially important as a first line of defence against tumor cells.

Using the morphology of the human NK cells as markers we have improved the fractionation methods and obtain 85-90 percent pure fractions of such cells for continuation studies.

The presence of NK cells within human solid tumors has been investigated and the program on the specific immunological characteristics of normal and neoplastic glial cells continued. Various other tumor markers have been investigated and also applied to clinical diagnostic work.

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337. UVEITIS - SPECIFIC AND NONSPECIFIC CELLULAR REACTIVITY

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Alterations in cellular and humoral immunity have been described in both uveitis and ocular tumors; the biologic importance of these alterations is unclear. Is reactivity towards ocular antigens important in the pathophysiology of these diseases or is it an epiphenomenon secondary to nonimmunologic alterations of tissue antigens?

Circulating immune complexes have been demonstrated in both animal models and human diseases. Preliminary evidence indicates that some uveitis and ocular tumor patients have elevated levels in immune complexes. Currently studies are ongoing to characterize the nature of the antigen and antibodies in these complexes and use the separated antigens to study specific humoral and cell mediated immunity in these diseases.

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338. FACTORS INFLUENCING METASTATIC NEOPLASIA IN THE CENTRAL NERVOUS SYSTEM

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The Neurosurgery Research Laboratory has continued work defining parameters which influence the nonspecific cellular immune response against metastatic brain tumor produced by chronic infection with Toxoplasma gondii in mice. (See JNCI 63: 1237-1244 1979). Complete tissue culture facilities were in operating condition by March 1980, and we now have the capability of growing tumor cells in culture, free from contamination by host cells. We have successfully labeled cells with 125IUDR using a dose of isotope which tags 90% plus of the cells yet does not interfere with the ability of the tumor cells to grow into multiple metastatic tumor nodules. Experiments are just under way which will follow the fate of intracardially injected 125IUDR labeled KHT tumor cells in control, T. gondii infected and C. parvum-treated mice using radioactivity counting and autoradiography of tissue sections. A study of the effects of anticoagulation on metastatic tumor is also in progress. During this past year we have done extensive work developing an immunocytochemical method to demonstrate Toxoplasma antigen in formalin-fixed, paraffin-embedded tissue sections. A study of human cases of necrotizing Toxoplasma encephalitis will be published in Human Pathology and a study looking at sequential CNS infection with Toxoplasma in mice has been submitted for publication. The method is presently being used by a number of investigators for the tissue diagnosis of Toxoplasmosis in immune compromised patients and experimental animals.

339. HUMORAL CYTOTOXIC RESPONSE IN GLIOMA PATIENTS

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The humoral immunity of patients with malignant tumors of the central nervous system is being studied using an established in vitro microcytotoxicity assay. A longitudinal study using both autologous and allogeneic tumor cells is under way to determine the frequency of complement dependent cytotoxic antibodies in patients with varying tumor loads. Sera from these patients will be fractionated into IgG, IgM and IgA classes to determine the immunoglobulin class of the cytotoxic antibodies and how the class might alter with changes in the biological behavior of the tumor. Patients with other tumors and non-neoplastic central nervous system disease as well as normal persons will be tested for the presence of these antibodies. Serial absorptions were carried out to determine the specificities of the cytotoxic response. The methodical examination of the humoral response of patients with central nervous system tumors will lead to refined techniques for the immunodiagnosis and serological monitoring of these patients.

340. PATHOGENESIS OF CNS AND AUTOIMMUNE DISORDERS

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During the coming year we plan to extend our analyses of the association of circulating immune complexes with human and experimental disease, including autoimmunities, vascular disease, malignancy, and the diseases of aging. We will follow up the indication that selective absence of IgA promotes absorbtion of antigens leading to autoimmunity and immune complex injury. We will investigate, in depth, evidence which indicates that dietary restriction from weaning inhibits development of diseases of aging including vascular sclerosis, autoimmunities and malignancy, and also inhibits thymic involution and progressive disorganization of lymphoid system and immunologic functions which otherwise occur with aging in short-lived autoimmune-prone mice.

Finally, we will determine whether evidence of central nervous system changes occurs relatively early with aging in short-lived autoimmune-prone mice and observe whether or not dietary restriction from weaning can inhibit these processes.

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341. AGE-DEPENDENT ALTERATIONS OF ADHERENT CELL FUNCTION

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We will study the role of macrophages, which appear to act as primary effector cells in conferring protection from lethal viral-induced central nervous system disease. Preliminary studies show that SJL mice exhibit an age-dependent resistance to cen-

tral nervous system infection due to the neurotropic JHM strain of mouse hepatitis virus. Resistance can be transferred from old (12 week) resistant mice to young (6 week) susceptible animals with either splenic or peritoneal macrophages taken from animals which exhibit no evidence of immunity to mouse hepatitis virus. Macrophages from 2 and 12 week old animals will be compared to determine if additional age-dependent changes in macrophage functions can be found. The peritoneal exudate cells from the two age groups will be compared for the number of total macrophages; the level of 'activation'; the numbers of Ia antigen, Fc, and C3 receptor bearing cells; and the ability to function as phagocytes, as ADCC effectors, and as T and B cell helpers. In addition, we will examine possible genetic restrictions conferred upon these cells in their role as primary effector cells and the possible interactions with other cells of the immune system.

342. IMMUNOLOGIC MECHANISMS OF CATTLE

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OBJECTIVE: Perform in vitro studies on cellular and humoral elements of the bovine immune systems with tissues provided by the Government from experimental animals involved in a study of immunoprophylaxis of bovine odular squamous cell carcinoma, in order to determine whether correlative changes in immune response can be detected during the process of cancer development, and if so, to determine the immunologic mechanisms involved. The work will be carried out in two phases. The first phase will represent a feasibility study for the development and selection of an appropriate number of relevant tests, subject to approval and acceptance by the Project Officer. The second phase will involve the application of the tests selected to the program objectives stated above.

343. HUMORAL ANTIBODY-MEDIATED AND MACROPHAGE-MEDIATED IMMUNITY IN A FELINE MODEL OF OCULAR MELANOMA

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No summary has been provided to the Smithsonian Science Information Exchange.

344. CIRCULATING IMMUNE COMPLEXES AND COMPLEMENT LEVELS IN NEUROBLASTOMA

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No summary has been provided to the Smithsonian Science Information Exchange.

C. IMMUNOBIOLOGICAL AND GENETIC STUDIES OF CNS TUMORS

345. BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF MALIGNANT NEUROGENIC TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM

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OBJECTIVE: In all patients with neuroblastoma, characteristic aberrations of the catecholamine metabolism are found. These aberrations are relevant to diagnosis but may vary from patient to patient. The question therefore arises whether it might be biochemically possible to differentiate, initially, between patients with a better and a poorer prognosis, even if clinically, e.g., by staging procedures, no difference can be found. As a rule younger patients with neuroblastoma have a better prognosis than older ones. Furthermore, neuroblastoma is one of the most important representatives of the fascinating group of malignant tumors that may regress spontaneously or may differentiate into a benign form (ganglioneuroma). Since these properties suggest that endogenous defense mechanisms exist, it is of interest to study the influence of immunologic factors on the growth and/or the behavior of neuroblastomas both in vitro and in vivo.

APPROACH: One of our goals is to search for more sensitive and more specific methods of the quantitative determination of catecholamines, their precursors and catabolites in biological material (urine, blood, tumor tissue) of patients with neuroblastoma. Another goal is the determination of immunoglobulin classes, subclasses and genetic markers in the serum of such patients. In addition, attempts are made to incubate human neuroblastoma cell lines with sera or isolated immunoglobulins obtained from patients in various stages or phases of the disease in order to search for specific antibodies by indirect immunofluorescence or to demonstrate antibody-dependent cellular cytotoxicity. The results of such studies are correlated with clinical data.

PROGRESS: The 2-year-survival rate of patients with predominantly dopamine-producing neuroblastoma appears to be clearly lower than that of patients with a predominantly noradrenaline-producing tumor. In addition, combinations of certain genetic markers of immunoglobulins, the Gm phenotypes Gm (a positive f positive g negative b positive) and Gm(a positive f positive g positive b negative) are significantly more frequent among patients with neuroblastoma than in adequately matched control groups.

346. IMMUNOBIOLOGY OF ASV INDUCED PRIMARY BRAIN TUMORS

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The overall objective of this proposed research is to establish the avian sarcoma virus (ASV) induced primary brain tumor in rats as an immunologic parallel to humans harboring morphologically and biologically similar brain tumors, using related quantitative and qualitative in vitro and in vivo immunologic probes. The proposed research is designed to assess immune function during the pre-tumor and tumor states. Lymphocytes from ASV inoculated rats will be quantitated via their cell surface receptors and their responsiveness to mitogens determined. Defects in macrophage function will be assessed employing a chemotaxis assay. The in vivo immune competence of ASV inoculated rats will be evaluated by measuring humoral antibody production and cutaneous skin reactivity at various times after immunization with particulate and soluble antigens. Antibody and cell mediated tumor immunity as measured by 51Cr release assays, mixed lymphocyte-tumor reaction and lymphocyte blastogenesis to neural (normal and neoplastic) antigens will be determined. The mechanisms for the diminished general immunocompetence observed in tumor-bearing rats will be sought by characterizing cellular and/or humoral suppressor factors. Spleen cells from these rats will be fractionated on glass bead columns and the responsiveness of these fractions to mitogens and to tumor and/or neural antigens determined. The identity of the suppressor cell(s) will be determined by employing reconstitution experiments together with abrogation of suppressor cell activity by specific antisera and, finally, further fractionation of the cells based on functional and cell surface receptor characteristics. Classical chemical and biophysical techniques will be utilized to study and isolate the tumor induced serum suppressor factor(s) using as a monitor of suppressor activity the responsiveness of lymphocytes to mitogens and neural antigens. Experiments will also be conducted to identify substances (serum factors, antigen-antibody complexes, tumor cells, etc.) from tumor-bearing rats which induce suppressor cells.

347. IMMUNOBIOLOGY OF PRIMARY INTRACRANIAL TUMORS

Roszman, T. L., Brooks, W. H., University of Kentucky, School of Medicine, Dept. of Pathology, *Limestone & Euclid, Lexington, Kentucky,* 40506, United States of America

The overall objective of this proposed research is to elucidate the unique relationship of the central nervous system (CNS) and the immune system as a 'network' in humans with primary malignant brain tumors. Further definition of the cellular mechanisms associated with impaired host immunocompetence of brain tumor patients will be determined by characterizing lymphocyte subsets via number and affinity of lectin surface

receptors and assessing alterations in translocation receptor movement and in stability of sheep erythrocyte receptors. The relationship of cyclic nucleotides and hormones with lymphocyte activation also will be studied in these patients. These quantitative and qualitative cellular changes will be correlated with alterations in host immunocompetence. Furthermore, the ability of various bran extracts, sera (fraction), and hormones to induce similar quantitative and qualitative changes in normal lymphocytes will be assessed. Classical immunochemical and biochemical techniques will be utilized to isolate and characterize immune-inhibitory fractions from tumor extracts and sera of tumor patients and correlated with changes in lymphocyte number and function as well as general and tumor associated immunity. Finally, these immunological probes will be sequentially correlated with the clinical course of each patient.

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348. QUANTITATIVE IMMUNE RESPONSES OF PATIENTS WITH INTRACRANIAL NEOPLASMS

Brooks, W. H., Roszman, T. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *Leestown Pike, Lexington, Kentucky*, 40507, United States of America

The objectives of this project are: 1) To elucidate the immunobiological response of patients harboring primary central nervous system neoplasms and 2) to define precisely the clinical usefulness of such sequential immunological assessments. Accordingly, pre-operative and subsequent sequential immunologic profiles are obtained on each patient by determining: a) cutaneous delayed-type responsiveness to common antigens and neoantigens; b) the percentage and absolute number of various lymphocyte subpopulations; c) lymphocyte responsiveness to non-specific mitogens; and d) the presence of humoral factors capable of abrogating various cellular reactions. To date, immunological profiles have been obtained from 82 patients and correlated with prognosis and survival. These results indicate that although isolated assessments are not helpful, sequential data can be utilized to construct an immune response index which does predict pre-clinical recurrence. Furthermore, investigations of the in vitro correlates of cell-mediated immunity in these patients have demonstrated a functional qualitative defect of lymphocytes obtained from patients which contributes to impaired host immunocompetence. In our efforts to understand the neural-immunological network, studies are being continued to define this plasma membrane defect precisely.

349. KILLER LYMPHOCYTE DAMAGE TO TARGET CELL MEMBRANES

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Electrical measurements of membrane potential and resistance of neuroblastoma and cultured muscle cells have been made using intracellular microelectrodes while the cells are undergoing immunologic attack by antibody and complement and by killer T lymphocytes. Membrane damage by complement is indicated by a rapid loss of membrane potential and resistance which occurs 20-30 minutes prior to cell death measured by dye uptake or phase microscopy. Alloantigen sensitized T lymphocytes specifically cause membrane depolarizations of 20-40 mv lasting 3-30 seconds within minutes after contact with the target cells with only minor changes in resistance. In some cases a complete and irreversible depolarization followed after 1-3 hrs. Another approach to examining such membrane damage has been to study the escape of markers from resealed erythrocyte ghosts being attacked by various lytic agents. A series of fluorescent proteins and dextrans of various molecular weights was resealed inside the ghosts; a sieving effect was revealed when small macromolecules but not large ones were released by complement and by an antibody dependent lymphocyte mediated process. Aqueous channels through the membrane having diameters of 50 A and 140 A for complement and lymphocyte killing respectively are suggested from the size cut-off of the protein markers.

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350. IMMUNE REGULATION AND GENETICS OF DISSEMINATED NEUROFIBROMATOSIS AND ITS MALIGNANT CONVERSION

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The incidence of neurofibromatosis and the factors leading to malignant conversion within those affected remain obscure. This prospective study aims to assess the genetic and immunological patterns associated with conversion to malignancy and any environmental factors which may contribute to it. Approximately 300 families from the eastern portion of the U.S. will be studied. Personal records of birth, work history, mortality, location, occupational exposures, and specific genetic factors (HLA types), fibroblast conversion, and immunological factors (immune complexes) associated with malignant transformation will be included in the analyses. The control group will be family members without neurofibromatosis. A National Neurofibromatosis Foundation has been started and ultimately may be used to contribute families.

351. THE BIOLOGY OF HUMAN NEUROBLASTOMA

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New and established human neuroblastoma cell lines will serve as the basis for a multifaceted study of a unique human tumor. This neoplasm, which can be present at birth, is known to regress spontaneously and to differentiate into a benign tumor. The various investigations will attempt to determine the role an immune response plays in the development and regression of the tumor and mechanisms involved in cellular control. The fields of investigation include membrane glycoproteins, mouse-human cell hybrids, human genetics, tumor specific transplantation antigens, cellular and humoral immune responses and their correlation with prognosis and response to treatment.

352. OCULAR LYMPHOID TUMORS - IMMUNOLOGY AND ULTRASTRUCTURE

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The grant focuses on the immunologic and ultrastructural evaluation of benign and malignant lymphoid tumors occurring in the conjunctiva, lacrimal gland, orbit, and other ocular tissues. The analysis of cases has shown that there are two types of lesions: immunologically polyclonal lesions, and immunologically monoclonal lesions. These two sub-types of lesions correlate closely with the routine light microscopic appearance, in that the former appear to have benign cytopathologic features, whereas the latter show evidence of immaturity or a monotonous population of borderline mature cells. In ultrastructural studies, it has been possible to identify nuclear irregularities in the malignant group, as well as a homogeneous population of cells in the malignant lesions in terms of similarity of cytoplasmic organelles and organization. In the reactive lesions, a higher percentage of mature lymphocytes, plasmocytes, and histiocytes has been observed.

353. IMMUNITY AGAINST NITROSOUREA-INDUCED RAT BRAIN TUMORS

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One hundred and eighty-seven glial malignancies have been generated in 128 offspring of 14 pregnant F-344 rats treated transplacentally with the resorptive, neurotropic carcinogen, ethylnitrosourea (ENU). In order to generate glioma lines for immunological experiments, 83 of such gliomas have been processed for (a) tumor cell cultures and (b) direct transplantation passages or alternate culture-to-animal passages to subcutaneous and intracerebral sites of syngeneic, sex-matched recipients.

Direct transplantation of ENU-induced gliomas to the intracerebral site of new hosts has yielded successful growths which have maintained the morphological features of parent glial tumors. Passage of ENU-induced gliomas to the subcutaneous site of new hosts either directly or by means of alternate culture-to-animal passage has yielded growths that, in a high proportion of cases, contain sarcomatous elements.

Immune reactions against ENU-induced gliomas in vivo and in vitro are being investigated and experiments are being designed to determine the source of sarcomatous elements in subcutaneous glioma transplants.

354. IMMUNOGENETIC CONTROL OF BRAIN TUMOR GROWTH IN RATS

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The rat gliosarcoma GS-9L is under immunogenetic control. It grows progressively in F344 rats and does not grow in rats of the strain KGH. After surgical removal of an established tumor in a susceptible F344 rat, rechallenge with a second tumor inoculation fails to produce tumor growth, indicating immunization by the tumor. 'Immune' lymphocytes from KGH animals confer resistance to the susceptible (KGH x F344)F1 hybrids in a Wienn Assay. An abstract was presented at the Reticuloendothelial Society meeting in December, 1979 and another abstract will be presented at the Federation of the American Societies of Experimental Biologists in April, 1980. A manuscript has been submitted to the journal, Cancer Research.

355. IMMUNOLOGICAL STUDY OF BRAIN TUMORS (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

356. IMMUNOLOGIC AND GENETIC ASPECTS OF RETINOBLASTOMA

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This is part of a broader project. A summary of this subproject is not available.

357. VIROLOGIC, IMMUNOLOGIC AND GENETIC ASPECTS OF MULTIPLE SCLEROSIS

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This is part of a broader project. A summary of this subproject is not available.

358. IMMUNODEFICIENCY IN PATIENTS WITH NEUROLOGICAL DISORDERS

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This is part of a broader project. A summary of this subproject is not available.

VI. STUDIES ON NERVOUS SYSTEM TUMOR GROWTH, DIFFERENTIATION, AND METABOLISM

A. ROLE OF CYCLIC NUCLEOTIDES AND POLYAMINES IN GROWTH AND DIFFERENTIATION

359. CYCLIC NUCLEOTIDES AND SPECIFIC NEUROCHEMICAL PARAMETERS IN BRAIN TUMORS

Racagni, G., Apud, A., Bruno, F., Giordana, M. T., Iuliano, E., Pezzotta, S., Ferrario, E., Universita degli Studi di Milano, Inst. of Pharmacology & Pharmacognosy, *Via A. Del Sarto 21, 20129 Milan, Italy*

OBJECTIVE: Cyclic AMP and cyclic GMP are involved in several cellular mechanisms and participate in the regulation of specialized functions of CNS as well as in the control of cellular growth and differentiation. Cyclic nucleotide systems are now regarded as intracellular second messengers mediating many actions of different hormones. As far as CNS tumors are concerned, it has been shown that stimulation of the cyclic AMP biosynthesis in cultured murine neuroblastoma cells dramatically decreases cell division. On the other hand, cyclic GMP system seems to be enhanced in neuroblastoma cells in culture. Besides cyclic nucleotide systems, other biochemical parameters may be altered mainly in the tumors of glial cells; among these, GABA seems to be of interest since this amino acid neurotransmitter presents a specific uptake system at the level of glia.

APPROACH: The present program deals with the study of cyclic nucleotide and GABAergic systems in different brain tumors induced in vivo by ethylnitrosourea (ENU). The ENU model is considered to be useful for experimental brain tumor studies, since the development and the morphology of the tumors are similar to the human brain tumors. ENU induces brain tumors in the offspring of mothers treated intravenously with a low dose (20 mg/kg) between the 15th and the 17th day of verified pregnancy. The biochemical investigations will be performed during the different stages of tumor development and will be correlated with the morphological changes. Of particular interest will be the investigation of the morphological and biochemical changes during the earliest neoplastic proliferations. Apart from the in vivo studies, this program will also investigate the biochemical variations in culture cells of fetal brain derived from ENU treated mothers; furthermore, cyclic AMP, cyclic GMP and GABA concentrations in the CSF of patients bearing nervous system tumors will be examined.

360. THE ROLE OF THE MULTIENZYMATIC SYSTEM OF CYCLIC NUCLEOTIDES IN THE GROWTH OF HUMAN AND EXPERIMENTAL BRAIN TUMORS

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Considering the importance of cyclic nucleotides (cAMP and cGMP) in modulating cellular growth (both in normal and neoplastic tissue) we have studied the metabolism of cyclic nucleotides in human tumors of different histological type and malignancy.

APPROACH: The project will deal with the following metabolic steps: a) Study of adenyl cyclase and of guanosyl cyclase as well as of phosphodiesterase (PDE) (acting on either nucleotide) in brain tumors. Data so far obtained indicate that both cyclase and PDE are lowered in neoplastic tissue. b) Study of the endogenous protein activator of PDE in brain tumors. c) Study of protein kinase in brain tumors. This part of the program is now under investigation: preliminary results are briefly reported in the next paragraph.

Protein kinase (PK) activity has been investigated both in cytosol and in particulate fraction, in basal conditions and after stimulation with cyclic nucleotides. In basal derivation and malignancy. In neurinoma the basal PK is significantly stimulated in cytosol by both cyclic nucleotides (cAMP and cGMP). On the other hand, in glioblastoma and in astrocytoma, which are

tumors of more pronounced malignancy, cGMP specifically induces a remarkable increase in PK activity, and this biochemical event is signficantly present only in the particulate fraction which is more important for the physiological activity of PK.

In tumoral tissues the ratio cAMP/cGMP dependent PK both in cytosol and in particulate fraction is decreased, in comparison with normal gray and white matter, indicating that tumors have a higher activity of the cGMP-dependent PK. It is important to note that only the ratio measured in the particulate fraction of glioblastoma and astrocytoma demonstrates a PK stimulation by cGMP higher than by cAMP, completely reverting the normal ratio pattern.

361. RELATIONSHIP BETWEEN POLYAMINES AND CYCLIC NUCLEOTIDES - CONTROL MECHANISMS OF NORMAL AND NEOPLASTIC GROWTH

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The naturally occurring polyamines, spermine, spermidine and putrescine, seem to play an important role in the control mechanisms of macromolecular synthesis and cell division. The activity of ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine biosynthesis, increases shortly after a stimulus inducing cell division or after increased cellular cyclic AMP accumulation, following hormone stimulation. The rationale of this effect of cyclic AMP is not clear also from the point of view of the inverse relationship between this factor and the rate of cell proliferation. The aim of this project is to verify a possible involvement of polyamine itself in the control mechanism of cellular cyclic AMP content, and therefore to determine if the cyclic-AMP mediated induction of ODC activity and polyamine accumulation may represent an important step of a self-regulatory mechanism triggered by the increased cyclic AMP. For this purpose, first of all we will study the effect of exogenous polyamines on cyclic AMP content of different types of cultured cells, both normal (myocardial cells and fibroblasts) and neoplastic (neuroblastoma, glioma and neuroblastoma-glioma hybrid cells). In parallel, the effect of specific inhibitors of polyamine synthesis on cyclic AMP will be considered as well as the effect of exogenous polyamines on the action of different hormones or chemicals all inducing cyclic AMP accumulation. On the basis of preliminary results indicating that exogenous polyamines, at relatively low concentrations, are able to decrease the intracellular cyclic AMP content probably by affecting cyclic AMP-dependent phosphodiesterase activity, we will study the effect of exogenous polyamines on cellular phosphodiesterase, both soluble and particulate, as well as on the high and low Km activities. Finally a possible role of polyamines in phosphodiesterase induction elicited by cyclic AMP will be investigated by studying the sequential events that follow the treatment of cell cultures with promoters of cyclic AMP accumulation, in presence or absence of a specific inhibitor of ODC activity, as alpha-methylornithine.

362. CULTIVATION OF HUMAN CANCER CELLS IN VITRO - FUNDAMENTAL AND APPLIED STUDIES

Sekiguchi, M., Hirono, H., Ishii, S., Kamada, N., Kosaka, H., Minato, K., Miyamoto, T., Miyoshi, I., Nishihira, T., Notake, Y., Kasuga, T., Suzuki, T., University of Tokyo, Inst. of Medical Science, Dept. of Clinical Oncology, *P.O. Takanawa, Tokyo, Japan*

OBJECTIVE: Establishment of continuous cultured cell lines derived from human carcinomas has been infrequently successful by chance. We are trying to cultivate such human cancer cells which have so far been difficult to establish as cell lines. When cell lines are obtained, they are characterized and utilized for fundamental and applied studies of human cancer.

APPROACH: The following three directions of applied research are especially emphasized. (1) Studies of mechanism of cell differentiation which will provide information applicable to cancer treatment. (2) Establishment of reliable systems in cell culture for evaluation of effectiveness of anticancer drugs. (3) Immunological studies, including extraction of cancer-specific antigens from cultured cells and their characterization, and related investigations of tumor immunity.

PROGRESS: (1) Addition of cAMP in culture medium induced inhibition of cell proliferation and increased melanin synthesis in melanoma cell lines, and inhibition of cell proliferation, elongation of cytoplasmic processes, and network formation in neuroblastoma cell lines. Administration of cAMP to nude mice inoculated with cultured neuroblastoma cells or melanoma cells inhibited tumor growth. Purified cholera enterotoxin, which is known to increase cellular cAMP, induced network formation of neuroblastoma cells in vitro. Growth of heterotransplanted human neuroblastoma and melanoma in nude mice was inhibited by intratumoral injection of this substance. Thus, tumor regression due to cell differentiation was successfully induced by cholera toxin, although it is highly toxic. (2) Screening of anticancer drugs by the use of cultured human cancer cells is a promising application of cell culture. However, there are many technical difficulties. Evaluation by colony forming ability in soft agar is the most reliable, but it is laborious and time-consuming. Evaluation by drug concentration giving 50% growth inhibition differs widely depending upon kinds of drugs, cellular growth rate, incubation time, and timing of evaluation. Evaluation by regrowth assay at 90% lethal dosis is satisfactory and reproducible; cultured cells derived from poorly differentiated gastric cancer were much more sensitive to mitomycin-C than those from well differentiated gastric cancer. (Text Abridged.)

363. BIOCHEMICAL STUDIES OF DIFFERENTIATION IN CULTURED NORMAL AND NEOPLASTIC CELLS OF NEURAL ORIGIN

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OBJECTIVE: Dbc-AMP or agents that enhance cAMP formation induce morphological and biochemical differentiation of various tissue cultured cells. The nature of this process is studied in glioma (138 MG) and neuroblastoma (C 1300) cells and compared with the spontaneous differentiation of cultured astroblasts from brain hemispheres of newborn rats.

APPROACH: The cAMP-system is studied with respect to cAMP levels, activation of adenylate cyclase and phosphodiesterase activity during the differentiation process. Its relation to changes in high energy phosphates and ATPase activity is described.

Indirect immunofluorescence is used for visualization of changes in the cellular fibrillary structure, in particular the distribution of microtubules (MT) and glial intermediate filaments (GFA).

PROGRESS: The differentiation is partly linked to activation of the cAMP-system and is manifested in changes in energy metabolism, ATPase activity and reorganization of structural proteins.

364. EFFECT OF MORPHINE AND RELATED OPIATES ON CYCLIC AMP-DEPENDENT PROTEIN KINASE AND ORNITHINE DECARBOXYLASE ACTIVITIES IN HYBRID CELLS (ABBREV)

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OBJECTIVE: It has been well established that morphine inhibits adenylate cyclase activity in neuroblastoma x glioma hybrid cells and that drug tolerance is followed by an increase in the synthesis of adenylate cyclase in these cells. This enzyme which is partially inhibited in the presence of the opiate, is activated after removing the drug, thus leading to the biochemical changes related to the withdrawal process. It is conceivable that changes in adenylated cyclase activity lead to a cascade of events which may include modulation in the activities of cyclic AMP-dependent protein kinase and ornithine decarboxylase.

APPROACH: Neuroblastoma x glioma hybrid cells (NG 108-15) will be used. The intracellular levels of cyclic-AMP, cyclic AMP-dependent protein kinase and ornithine decarboxylase will be determined during the acute phase of cell exposure to morphine, as well as during the induction of opiate tolerance and withdrawal.

365. ROLE OF POLYAMINES IN MAMMALIAN CELL PROLIFERATION

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Although high levels of polyamines, viz., putrescine, spermidine and spermine, are associated with rapid growth and cell division in eukaryotic systems, relatively little is known about their role in growth regulation. The recent discovery of specific inhibitors of polyamine biosyntheis, such as methyl gloxal bis (guanylhydrazone), alpha-methyl ornithine, and 1, 3-diaminopropane has opened up a new chapter in understanding the relationship between polyamine levels and other cellular functions. It is now well established that polyamines are essential for DNA synthesis in mammalian cells. Our preliminary studies with methyl glyoxal bis (guanylhydrazone) on HeLa and Chinese hamster ovary cells revealed that depletion of spermidine and spermine levels affect not only DNA synthesis but also chromosome condensation, cytokinesis, regulation of nuclear division and in vitro differentiation of mouse neuroblastoma cells. Under this project we propose to pursue these leads in a detailed and systematic fashion using the techniques of cell fusion, premature chromosome condensation, scanning and transmission electron microscopy for ultrastructural studies of prematurely condensed chromosomes and immunoelectronmicroscopy to determine the role of polyamines in the structure and function of microfilaments. We also propose to study the molecular basis for the relationship between the depletion of polyamines and the inhibition of DNA synthesis. The role of polyamines in the regulation of nuclear division in normal and transformed cells will also be studied. The results of these studies may provide some clues as to how we could interrupt the division cycle of tumour cells by disrupting the biosynthesis of specific polyamines. A better understanding of the roles of polyamines at various points in the mammalian cell cycle may prove to be helpful in the control or treatment of human malignancies.

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366. METABOLIC REGULATION IN GLIOMA CELLS

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The components of tachyphylaxis in the cAMP response to beta-adrenergic agonists by C-6 astrocytoma will be characterized. The components of tachyphylaxis include an induction of cyclic nucleotide phosphodiesterase and decreased hormone stimulated adenylate cyclase activity. Once the phosphodiesterase and receptor-adenylate cyclase changes are characterized in astrocytoma cells their significance in brain function will be analyzed. Induction of cyclic nucleotide phosphodiesterase will also be evaluated in human leukocytes.

Another phase of the project will study cAMP mediated protein phosphorylation in intact cells. The goal of these experiments is to enumerate the phosphoproteins which are either phosphorylated or dephosphorylated as a result of cAMP action. Subcellular fractionation will then be employed to assign the relevant phosphoproteins to subcellular compartments. Once the subcellular compartmentation is understood, individual proteins will be studied to determine the function of each.

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367. CYCLIC AMP AND METABOLIC CONTROL IN THE NERVOUS SYSTEM

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The aim is to obtain detailed understanding of changes in brain structure and function mediated by cAMP, cGMP and a specific Ca(plus plus) binding protein (CDR). The role of cGMP in the regulaton of gene expression will be examined. The possibility that the defects associated with Down's syndrome may be secondary to disordered or excessive cGMP during development will be analyzed, noting particularly the possible involvement of superoxide dismutase in activation of guanylate cyclase. The heat shock phenomena of Drosophila cells and of mammalian fibroblasts in culture provide model systems which will be utilized in studies of cGMP and altered patterns of protein synthesis and post-translational modification. Experiments designed to reveal interactions between Ca(plus plus), CDR, cAMP, and cGMP will be conducted using intact cells of the C-6 glial tumor, primary cultures of glia and nerve ending particles. Actions of neurotransmitters, GTP and CDR are of particular concern. Possible cGMP-mediated effects on nuclear phospho-proteins will be analyzed.

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368. BIOCHEMICAL EVENTS IN DIFFERENTIATING NEUROBLASTOMA

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Neuroblastoma, a common solid tumor of children, has been treated with limited success with cytotoxic chemotherapeutic drugs. It may spontaneously differentiate in vivo, perhaps preventing tumours from presenting themselves. In model studies, clonal mouse neuroblastoma cells grown in tissue culture with dibutyryl cAMP or other agents increasing intracellular cAMP levels are not only biochemically and morphologically differentiated but are less tumorigenic in the syngeneic Ajax mouse. We propose to investigate the correlation between differentiation, as evidenced by increases in the levels of specific markers including cAMP binding proteins, and their in vivo tumorigenicity. The transcriptional, translational and post-translational mechanisms will be studied including the effectors of transcriptional control, the nuclear acidic proteins and the changes in the synthesis and processing of m-RNAs. Control at the translational level will be studied by the analysis of levels of the in vivo synthesis of specific proteins, separated by two-dimensional gel electrophoresis, and extended by analysis of the levels of cytoplasmic messenger RNAs assayed in in vitro protein synthesizing systems. We will also investigate the altered synthesis and/or glycosylation of membrane proteins as the direct modulators of tumorigenicity. Specific alterations which correlate with reduced tumorigenicity will define a system in which to predict and test new non-toxic drugs capable of differentiating neuroblastoma in vivo.

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369. REGULATION OF NERVE GROWTH FACTOR CONTENT OF NERVOUS SYSTEM-DERIVED TISSUES

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Nerve growth factor (NGF) is synthesized and secreted by two cell lines derived from the nervous system, the rat C6 glioma and the mouse NB2a neuroblastoma. The content of the

NGF is increased in either cell line by agents which elevate cyclic AMP, beta-adrenergic receptor agonists for the glioma cells and prostaglandin E1 or adenosine for the neuroblastoma. The increase in NGF content follows an elevation of cyclic AMP content and the concomitant activation of protein kinase. The increase in cyclic AMP does not depend on an increase in the net synthesis of NGF, but rather appears to derive from an activation of a peptidase which may form NGF from a pro NGF. Research is being carried out to determine 1) the characteristics of the glioma cell protein kinase(s); 2) the precise mechanism by which protein kinase activates the peptidase and 3) the biochemical characteristics of the precursor NGF.

BIBLIOGRAPHIC REFERENCES: Schwartz, J.P., and Costa; E.: Regulation of nerve growth factor content in a neuroblastoma cell line. Neuroscience 3: 473-480, 1978.

370. PROTEIN KINASE TRANSLOCATION AND GENE EXPRESSION

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There is evidence indicating that cAMP (adenosine 3', 5'-monophosphate) mediates the induction of protein synthesis at the transcriptional level. Cyclic AMP regulation of transcription conceivably is mediated through a mechanism involving nuclear translocation of cytoplasmic cAMP-dependent protein kinase, phosphorylative modification of nuclear regulatory proteins resulting in altered DNA template activity, altered RNA polymerase activity, and altered RNA synthesis, that is the synthesis of the specific mRNA which codes for the induced protein.

The proposed studies utilize the C6 glioma cell to define (a) the precise mechanism of nuclear translocation of protein kinase by biochemical and immunochemical methods; (b) the role of nuclear translocation of cytoplasmic protein kinase in the phosphorylative modification of nuclear nonhistone proteins; (c) the role of protein kinase translocation in the induction of isoproterenol-stimulated lactate dehydrogenase synthesis and synthesis of lactate dehydrogenase mRNA.

BIBLIOGRAPHIC REFERENCES: Christensen, M., Schweppe, J.S., and Jungmann, R.A. (1979) Cyclic AMP-dependent and -independent Protein Phosphokinase Activity in Subcellular Fractions of Synchronously Growing Leydig I-10 Cells. Exptl. Cell Res. in press. Mednieks, M.I., Jungmann, R.A., and Schweppe, J.S. (1979) Ontogeny and Multiplicity of Cyclic AMP-dependent Protein Kinase in Thymic Lymphoid Cells. Arch. Biochem. Biophys. in press.

371. IN VITRO STUDIES ON HUMAN NEUROBLASTOMA CELLS

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Neuroblastoma is the third most common malignancy affecting children. In spite of the application of combination chemotherapy to this childhood cancer, the survival rate of children with disseminated disease has shown little improvement. An interesting aspect of this particular cancer is its rather high rate of spontaneous differentiation to benign ganglioneuroma in the absence of treatment. Differentiation has also been noted to occur in tumors in children following chemotherapy.

We have recently established, from a patient with neuroblastoma, a cell line that consists of a mixed population of both undifferentiated and relatively differentiated cells. Current efforts are aimed at separating and characterizing these subpopulations. In culture, this cell line can be induced to undergo morphological differentiation by dibutyrylcyclic-AMP but not by a range of other agents which can nevertheless induce differentiation of mouse neuroblastoma cells. Further work is in progress to determine whether this process is complemented by biochemical differentiation in terms of catecholamine production.

372. EFFECTS OF ANTICONVULSANTS ON CYCLIC NUCLEOTIDES IN CULTURED CELLS

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Cultured mouse neuroblastoma cells were used to determine the effects of anticonvulsants, particularly phenytoin, on cyclic GMP and membrane electrical events. Neuroblastoma cells respond to depolarization (as well as to activation of certain membrane receptors) by increasing cyclic GMP synthesis in a manner apparently similar to that seen in brain slices. We have found that phenytoin and other anticonvulsants active against major seizure types inhibit cGMP increases in neuroblastoma cells by a mechanism involving blockade of calcium influx or translocation. This property is not shared by drugs which are active only against petit mal seizures. These findings may help provide a basis for understanding the biochemical changes associated with convulsive activity and the mechanism of action of anticonvulsant drugs.

373. ADENYLATE CYCLASE REGULATION IN NEUROBLASTOMA CELLS

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This study examines the neuron's response to the simultaneous presence or sequential appearance of multiple neuromodulators. My approach is to investigate the determinants of adenylate cyclase activity at the level of the plasma membrane of cultured neuroblastoma cells. This multicomponent enzyme synthesizes cyclic AMP, a second messenger that regulates intracellular functions. Cell surface receptors for prostaglandins are stimulatory regulators of adenylate cyclase; receptors for muscarinic agonists or opiates are inhibitory regulators. The goal of these studies is to determine the mechanisms of receptor mediated inhibition and the ensuing adaptive response of the cells to chronic stimulation by inhibitory agonists. It is hoped that these experiments will advance our understanding of how neurons in the brain 'learn' to respond to normal synaptic input and aid in the development of tolerance drugs.

374. FUNCTIONAL REGULATION OF CLONAL NEURONAL CELLS

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The proposed research will study the regulation of cellular function using the neuroblastoma x glioma hybrid cell, NG108-15, as a model system. The biochemical parameters to be studied will include receptor binding activities, membrane-associated enzyme activities, neurotransmitter uptake processes and neurotransmitter releasing systems. The first part of the study will examine the regulation of these biochemical systems during differentiation induced by dibutyryl cAMP and other chemicals, and also during long-term exposure to neurotransmitters and drugs. In the second part of the research, the role of membrane lipid composition in the normal functioning of the cells and in their adaptive responses to environmental changes (i.e., dibutyryl cAMP, drugs, neurotransmitters) will be studied using membrane lipid modification techniques with intact cells. The effects of altering the acyl chain composition on differentiation, the biochemical parameters described above and also the electrical properties of the cells will be determined. By studying many different regulatory events in a single homogeneous cell population, insights will hopefully be gained into the mechanisms by which cellular function is regulated.

B. ROLE OF HORMONES AND OTHER HUMORAL FACTORS IN GROWTH AND DIFFERENTIATION

375. CELL SURFACE CHANGES IN MALIGNANT GLIA AND THE ROLE OF GLUCOCORTICOIDS

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OBJECTIVE: To determine whether malignancy in glial cells is correlated with specific cell surface changes and whether these can be influenced by glucocorticoids so that the cell surface reverts to a more normal state; in particular, whether it can be shown that cell surface modifications correlate with invasiveness and that steroid induced changes inhibit invasion.

APPROACH: Normal and malignant lines of human glia will be characterized to establish their astrocytic and neoplastic identity. Subsequently membrane preparations will be analyzed by fractionation of whole membrane and protease digests of the cell surface. The effect of glucocorticoids on whole membrane preparations and extracellular protease digests from normal and malignant astrocytes will be analyzed by gel filtration and two-dimensional electrophoresis in an attempt to identify specific alterations correlating with neoplasia. Cultures will then be examined to determine whether there is a trend towards the normal glial constitution of the membrane druing glucocorticoid induced cytostasis. If there is an indication of cell surface modification then invasion of normal tissue in organ culture will be examined to determine whether these modifications affect the infiltrative capacity of the cells. Finally the specific activity of cell surface fractions following hormone treatment and evidence for changes in the rate of degradation by extracellular proteases will be examined to determine whether changes in cell surface glycoprotein arise by de novo protein synthesis, glycosylation, or a change in the rate of protease dependent degradation.

PROGRESS: (a) Specific staining by immunoperoxidase technique has confirmed the presence of an astrocytic marker, glial fibrallary acidic protein (GFAP) in human glioma cultures. GFAP is also inducible with beta-methasone and cAMP. (b) Steroid binding experiments have demonstrated the presence of a high affinity glucocorticoid receptor of similar Kd and concentration to those of normal glia.

376. MITOGENS, ANGIOGENESIS AND TUMOR GROWTH

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A variety of tissue growth factors stimulate the growth of normal as well as neoplastic cells. In turn neoplastic cells elaborate factors which stimulate growth of normal cells. This proposal seeks to: I. Identify and characterize brain and pituitary growth factors which influence growth of normal and neoplastic cells. II. Identify and characterize growth factors in breast cancer cells which influence the growth of fibroblasts and possibly the growth of the neoplastic cells themselves.

APPROACH: Growth factors in brain, pituitary and breast cancer extracts will be purified using conventional chromatographic procedures and the mitogenic activity identified by an increase in thymidine incorporation or an increase in cell member using a variety of tissue culture techniques. After the purification of each, specific radioimmunoassays and receptors assays will be developed to be used to determine the tissue distribution, secretion and hence blood level of each growth factor. The effect of growth factors on the growth of neoplastic cells will be determined. Receptors for each growth factors will be sought in a variety of neoplastic cells.

377. STUDIES ON GROWTH CONTROL EXERTED BY THE PROTEIN NERVE GROWTH FACTOR ON PHEOCHROMOCYTOMA CELLS (CLONE PC12)

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OBJECTIVE: Understanding the molecular mechanism(s) by which the protein nerve growth factor (NGF) blocks division and induces differentiation in a neuronal population of rat pheochromocytoma (clone PC12) cells.

APPROACH: Characterization of the interaction between NGF and PC12 cells (receptor); internalization and intracellular fate of NGF; identification of cytoplasmic structural components where NGF is located (nucleus, contractile proteins, etc.); molecular changes occurring on the cytoplasmic membrane during NGF-induced arrest of mitosis and neurite growth.

PROGRESS: There are specific receptors for NGF in PC12 cells having Kd of 2 to 10 to the minus 10th power M; after binding NGF is internalized and concentrates around the nucleus; internalization of NGF is influenced by cell density; with confluent cells internalization is blocked; half-life of NGF in PC12 cells is 18-20 hours; NGF stimulates synthesis of new receptors by 6-8 fold within 3 days.

378. PROPERTIES OF HUMAN CELLS IN CULTURE

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Project is divided into three parts: A. Growth control in normal and neoplastic cells. B. Properties of malignant lymphoma and myeloma cells. C. RNA virus in tumor cell lines. The studies are concerned exclusively with human normal and malignant cells in vitro. The major aims are a) an understanding of the normal proliferation control; its dependence on growth factors and internal cell surface specialization, b) the breakdown of multiplication control in malignant glioma cells, c) role of EBV in Burkitt's lymphoma, d) the biology of histiocytic malignant lymphoma, e) use of cell surface markers for improved diagnosis of lymphoma, leukemia and myeloma and f) elucidation of the etiological and pathogenetic importance of an apparent C-type RNA virus in histiocytic lymphoma and myeloma lines.

379. MEMBRANOGENESIS IN CRETINISM AND HYPERTHYROIDISM

Timiras, P. S., Einstein, E. R., Hudson, D. B., Valcana, T., Vaccari, A., Draves, D. J., Carpenter, A. C., Einstein, E. R., Miller, C., Skochko, S. L., University of California, Berkeley Campus, School of Letters & Science, Dept. of Physiology & Anatomy, *Berkeley, California,* 94720, United States of America

In our previous studies we have demonstrated that alterations in the thyroid state induced neonatally in the rat, result in significant developmental abnormalities correlated, at the neurochemical level, with altered brain protein synthesis, altered composition of some specialized neural membranes (e.g., myelin, synaptic vesicles) and altered number and characteristics of thyroid hormone receptors in discrete brain regions and in liver. Our current studies have followed essentially three major directions, each a direct continuation of our original research: 1) to investigate the regulatory role of thyroid hormones in brain development and behavior by studying the effects of these hormones on number and characteristics of monoamine (NE, DA and 5-HT) receptors in discrete brain areas, at specific ages and in male and female rats; 2) to extend our studies from normal neural tissue in vivo to cultured brain tumor cells in order to establish whether thyroid hormones similarly regulate structural (e.g., microtubule polymerization), functional (e.g., ATPase activity) and receptor distribution in these tumor tissues. Furthermore by comparing neuroblastoma and glioma cells we may draw some conclusions as to the differential actions of thyroid hormones on neuronal and glial cells; finally 3) to study fatty acid composition and fluidity of biomembranes (especially mitochondrial membranes most susceptible to thyroid hormones) in the myocardium and the brain of adult rats (more suitable for methodologic reasons to these experiments than developing rats) to obtain a better understanding and a unified explanation for the multiple metabolic and functional actions of thyroid hormones in the myocardium, brain and possibly other tissues.

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380. NERVE GROWTH FACTOR IN THE CENTRAL NERVOUS SYSTEM

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Data from our laboratory have shown that nerve growth factor is synthesized and secreted by a variety of normal and transformed cells in culture. The function of cell-derived nerve growth factor is not known although it is possible that peripheral tissues produce the protein to supply sensory and sympathetic neurons. Recent studies indicate that central nervous system glioma cells as well as skeletal muscle cells also produce a form of nerve growth factor in culture. The cells interact with brain and spinal cord neurons and it could be that nerve growth factor has functions in the central nervous system.

Using chemical and biological methods, we propose to: 1) collect, isolate, and characterize the nerve growth factor which is produced by glioma cells and skeletal muscle cells; 2) assay the effects of these substances on the development of brain and spinal cord neurons in culture; 3) begin to develop histochemi-

cal techniques by which nerve growth factor can be localized in neural and non-neural tissues.

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381. PLASTICITY OF CHROMAFFIN AND PHEOCHROMOCYTOMA CELLS

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Pheochromocytoma cells in vitro may show morphological and functional properties of classic neurons and of classic endocrine cells. 'Neural' properties include neurite-like processes and small vesicles, choline acetyltransferase, specific antigens and sodium action potential channels. 'Endocrine' properties include large 'chromaffin' granules and peptide hormones. It is not clear to what extent ability to express these properties reflects normal pluripotency of 'neuroendocrine programmed' cells, and to what extent it is a manifestation of neoplasia. Background studies indicate that nerve growth factor (NGF) may cause pheochromocytoma cells to cease dividing and acquire morphological and functional characteristics of neurons, and also suggest that normal chromaffin cells remain responsive to NGF in adult life. Normal and neoplastic chromaffin cells may also be influenced by epidermal growth factor (EGF) and corticosteroids.

The proposed study will seek to identify interactions between NGF, EGF and steroid hormones which regulate 'neural' and endocrine' phenotypic markers and cell division in neoplastic, hyperplastic and normal adrenal chromaffin cells in culture, and to determine whether specific responses to these agents or to culture conditions may be correlated with neoplastic progression. The molecular level at which these agents exert their effects will be investigated by use of inhibition of transcription, translationa and replication. The role of cell-substratum adhesion in initiating or modulating effects of NGF and EGF will be studied by comparing effects of these growth factors in spinner and monolayer cultures. Mechanisms for storage and release of peptide hormones by chromaffin cells will be compared to known mechanisms for catecholamines. It is hoped that these studies will clarify events which determine normal neuroendocrine cell phenotype, and which are involved in the pathogenesis of neuroendocrine neoplasia.

382. GLANDULAR AND EXTRA-GLANDULAR NERVE GROWTH FACTOR

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We seek to determine whether the nerve growth factor (NGF) produced by the submaxillary gland of mouse is an important source of NGF for maintenance of the peripheral nervous system or whether other sources are of primary importance. If the latter is true, we propose to locate the important extra-submaxillary glandular sources and purify and characterize the NGF from these sources to determine whether NGF exists as 7S NGF or as some other molecular form.

We also propose to characterize the NGF-like activity of C6 glioma cells to determine whether it may actually be a factor other than NGF and to screen a large number of tissue culture cell lines to determine which types of cells may secrete NGF. If a cell line is found which secretes sufficient amounts of NGF, the NGF will be purified and characterized.

383. PURIFICATION OF ENDOTHELIAL PROLIFERATIVE FACTOR AND CHARACTERIZATION OF NORMAL GLIA

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Purification of the endothelial proliferation factor 'EPF' has proceeded utilizing the physico-chemical properties of the molecule. This approach has allowed 1) stabilization of the factor thereby facilitating further characterization, 2) determination of an approximate molecular weight, 3) determination of an approximate isoeletric point. Tumor cell conditioned medium, obtained from roller bottle cultures is fractionated initially using hollow fiber molecular sieves with molecular weight cut-offs of 100,000, 50,000 and 5,000 daltons. The mitogenic activity resides in the less than 100,000 and less than 50,000 dalton fractions. Rapid fractionation stabilizes the factor for six-month storage at 20 degrees C. The less than 100,000 molecular weight fraction has been subjected to preparative isoelectric focusing on granulated slab gels and has an isoelectric point of approximately 6.8-7.2. The terminal portion of the frog spinal cord is the frog filum terminale, which contains largely glia. This preparation has been used to establish the high affinity transport mechanisms for two neurotransmitter substances, gamma-aminobutyric acid (GABA) and serotonin (5HT). The uptake for both compounds is saturable, concentrative, sodium dependent and can be manipulated by chemical agents in precisely the same manner as the transport in tumor glia.

384. DIFFERENTIATION IN A MALIGNANT NEURAL TUMOR

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This project investigates the effect of glucocorticoids on the process of induced differentiation in neuroblastoma (using the C1300 murine model). Work on two aspects of this phenomenon is proposed. (A) The therapeutic effectiveness of glucocorticoids on tumors arising in A/J mice after inoculation with cultured neuroblastoma cells will be examined. Various dosages, schedules, and routes of administration of dexamethasone and similar steroids will be assessed by tumor incidence and latent period of tumor development. Tumors which develop will be profiled for DNA, RNA, protein and catecholamine content. Results may indicate whether dexamethasone treatment would be useful in treatment of neuroblastoma. (B) A demonstration and initial characterization of a cytoplasmic glucocorticoid receptor will be attempted using a competitive binding assay with 3H-dexamethasone as a radioligand. The affinity, saturability (and capacity), and specificity of a soluble protein complexing reversibly with glucocorticoids will be determined. This would serve as a start towards investigating the mechanism of action by which glucocorticoids induce differentiation in murine neuroblastoma.

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385. NGF AND DIFFERENTIATION OF NEURONAL TUMORS

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The regulatory mechanism for control of growth rate and differentiation of normal and neoplastic neuronal cells is poorly understood. Nerve growth factor (NGF) is a polypeptide important to the normal growth and development of the sympathetic nervous system of vertebrates. NGF can decrease the growth rate, induce neurite outgrowth, and enhance activity of enzymes regulating transmitter synthesis in rat pheochromocytoma (PC12) cells and in certain human neuroblastoma cell lines. The objective is to define the sequence of molecular events culminating in the differentiated state by studying binding of NGF, by identifying differentiation-specific macromolecular components (MC), by studying the pattern of MC in wildtype and NGF-resistant variants in the absence and presence of NGF, other agonists, and antagonists of neurite outgrowth. Attempts will be made to determine whether regulation of MC is at the transcriptional, translational or post-translational level. Binding of NGF to PC12 cells will be studied in culture and in vivo and will be compared with the properties of binding to rat adrenal chromaffin cells. Binding and response to NGF of cultured human neuroblastoma lines will be compared with that to freshly cultured tumors. There is weak circumstantial evidence suggesting involvement of NGF in neuronal tumors and this proposal is directed towards study and resolution of this possibility, as well as towards furthering the basic understanding of neuronal cell differentiation.

386. MATURATION FACTORS AND CANCER CELLS

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Glia maturation factor is a protein factor found in the adult brain that has the ability to enhance the phenotypic expression of normal immature astrocytes in culture. In the proposed work, experiments are planned to test the effect of the factor on various neoplastic glial cells. Morphological, biological and chemical parameters will be used to measure the effect. We are trying to determine if the factor blocks the appearance of some of the neoplastic properties of the cells in an otherwise permissive environment. It is hoped that the study will provide some insight into the interaction between the maturation factor and the malignant process.

387. CLONAL CELL LINES OF THE NERVOUS SYSTEM

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Myelination will be studied using primary cultures, purified oligodendroglia, and clonal lines exhibiting characteristics of oligodendroglia or Schwann cells. Following the onset and attainable levels of expression of a limited number of myelin-associated biochemical phenomena, especially the syntheses of myelin basic protein, proteolipid protein, and the levels of activity of 2', 3'-CpNase and cholesterol ester hydrolase, these studies will attempt to better define the environmental factors controlling myelinogenesis. These include the importance of extracellular matrix-cell and glial-neuronal interactions, exogenously supplied hormones and growth fators, and metabolic precursors.

388. HORMONAL STUDIES IN A CEREBRAL TUMOR (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

389. EFFECT OF CORTICOSTEROIDS ON BRAIN TUMOR WATER-ELECTROLYTE CONTENT (MICE)

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This is part of a broader project. A summary of this subproject is not available.

390. HYPOTHALMIC-PITUITARY FUNCTION IN EXTRAHYPOTHALMIC BRAIN NEOPLASIA

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This is part of a broader project. A summary of this subproject is not available.

391. INTERACTION OF MOUSE NERVE GROWTH FACTOR WITH HUMAN NEUROBLASTOMA CELLS

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This is part of a broader project. A summary of this subproject is not available.

392. DEMONSTRATION OF TRANSFORMING PROTEINS OF NEUROBLASTOMAS AND CARCINOMAS

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No summary has been provided to the Smithsonian Science Information Exchange.

C. VASCULAR ALTERATIONS IN TUMOR GROWTH AND MAINTENANCE

393. INVESTIGATION OF VASCULAR PERMEABILITY AND CEREBRAL EDEMA IN EXPERIMENTAL BRAIN TUMORS

Lantos, P. L., Deane, B. R., University of London, Inst. of Psychiatry, Dept. of Neuropathology, *Senate House, London WC1E 6BT, England, United Kingdom*

OBJECTIVE: A reproducible brain tumor model of short latency has recently been characterized in our laboratories. This model is a transplanted astrocytoma produced by intracerebral injection of cultured, cloned neoplastic astrocytes in the rat. It is currently being used in a study of the development of tumor vasculature. Structural and functional aspects of the blood vessels in and around the tumors are being investigated from the earliest signs of angiogenesis throughout tumor growth, with particular reference to the relationship between vasculature and: a) tumor cell proliferation and tumor malignancy, and b) the vasogenic edema associated with tumors and their abnormal vasculature.

APPROACH: The tumor vasculature and its structural abnormalities as compared with the blood vessels in normal adult brain have been characterized by light and electron microscopy. The permeability of the vascular endothelium to various specific substances is being investigated in detail, using electron-dense tracers in ultrastructural studies and radioactive materials in autoradiographic and in vivo studies on the formation and assessment of edema. Cytochemical studies on a variety of enzymes are also planned.

394. THE STUDY OF VASCULAR ALTERATIONS IN CEREBRAL AND CEREBELLAR METASTASES

Queiroz, A. C., Cunha Lima, C. R., Universidade Federal da Bahia, Lab. Anatomia Patologica, *c/o Hosp. Prof. Edgar Santos, Salvador, Bahia, Brazil*

OBJECTIVE: To study vascular alterations which develop in the nervous tissue peripheral to cerebral and cerebellar metastases, and to determine the significance of these alterations and possible pathogenic factors.

APPROACH: We have studied 24 cases of cerebral and cerebellar metastases. Histologic analysis of microscopic slides of the lesion is performed using hematoxylin, eosin, Masson trichrome, van Gieson and PAS stains.

PROGRESS: To date we have verified the occurrence of these alterations in 75.8% of all cases examined and in 100% of those that present with metastatic adenocarcinoma. We are currently studying the role of PAS-positive material in pathogenesis of the lesion. (Translated)

395. OCULAR ANGIOGENIC FACTORS - BIOASSAYS AND BIOCHEMISTRY

Glaser, B. M., D'Amore, P. A., Michels, R. G., Fenselau, A. H., Kimball, A. W., Johns Hopkins University, School of Medicine, Dept. of Ophthalmology, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

We propose to examine the hypothesis that the retinal, optic disc and iris neovascularization found in several pathologic ocular conditions occurs in response to a retina-derived angiogenic substance. To investigate this hypothesis we will continue our studies of human intraocular fluid samples. These samples are obtained from patients undergoing therapeutic vitrectomy for various ocular disorders in which retinal, optic disc and/or iris neovascularization is often a complication. The angiogenic activity of these vitreous samples will be studied using existing assays and this activity will be correlated with the clinical status of the eye.

Attempts to purify and characterize any angiogenic substances from human vitreal material is impeded, however, by the small quantity of this material available for study. To circumvent this obstacle we will continue the purification and characterization of an angiogenic substance (or substances) which we have recently identified in extracts of bovine, feline, rabbit and human retinas. Information obtained from the study of these more abundant materials will be used to guide the purification and characterization of angiogenic substances from human vitrectomy samples.

Eyes containing retinoblastoma, retina-derived tumor, are frequently found to have iris neovascularization. It is possible that a tumor-derived angiogenic substance may be responsible for the phenomenon. Furthermore, this substance may be related to the hypothesized retina-derived angiogenic substance. We will therefore continue ongoing studies of the angiogenic activity of conditioned media from human retinoblastoma cells in culture.

The various parameters affecting the liberation and action of human angiogenic substances are not readily amenable to experimental control. We will therefore attempt to develop an animal model in order to better study these parameters.

Currently available methods for determining angiogenic activity are somewhat limited in their specificity and sensitivity. We have therefore begun to develop two novel assays of angiogenic activity.

396. PURIFICATION OF ENDOTHELIAL MITOGEN OF CNS TUMOR ORIGIN

Haber, B., Suddith, R. L., Kelly, P., PerezPolo, J. R., University of Texas, Medical Branch at Galveston, School of Medicine, Dept. of Biochemistry, *300 University Blvd., Galveston, Texas,* 77550, United States of America

Central nervous system (CNS) tumors exhibit a high degree of neovascularization. The neovascularization of these and other solid tumors has long been recognized as necessary for tumor growth and maintenance. Several lines of evidence indicate that tumor neovascularization is mediated by a humoral factor elaborated by the tumor per se. We propose to use an in vitro model tissue culture system wherein human endothelial cells are the target of a trophic or vasoproliferative factor elaborated by tumor cells. Our previous work has shown that CNS tumor cells in culture elaborate a specific endothelial cell mitogen, termed by us endothelial proliferative factor (EPF). We propose to isolate and characterize this EPF from astrocytoma conditoned media, using the in vitro bioassay of endothelial proliferation. The astrocytoma cell line has been adapted to roller culture, which will insure the supply of large volumes of conditioned medium enriched in the endothelial mitogen. A long-term objective of the proposal is the production of monospecific antisera to EPF.

BIBLIOGRAPHIC REFERENCES: Suddith, R.L., Hutchison, H.T. and Haber, B. Uptake of biogenic amines by glial cells in Culture I. A neuronal life transport system for serotonin. Life Sci. Vol. 22, 1978. p. 2179-2188.

397. TRANSCAPILLARY EXCHANGE IN EXPERIMENTAL BRAIN TUMORS

Pollay, M., Lee, J. C., University of Oklahoma, Health Sciences Center, School of Medicine, Dept. of Surgery, *Box 26901, Oklahoma City, Oklahoma,* 73190, United States of America

The overall objective of this grant is to define the adaptive changes in blood-brain barrier (capillary endothelium) function that occur in central nervous system tumors and other pathological conditions affecting the nervous system. This will be accomplished by utilizing a technique that simultaneously measures regional cerebral blood flow and extraction of certain sugars, amino acids, and ketone bodies in transit through the cerebral capillary bed, and relating such changes in the kinetic parameters with changes in the morphology of the cerebral capillary. The major emphasis will be those changes that occur in the anatomy and physiology of the blood-brain barrier in a transplanted gliosarcoma in rat brain. The basic changes in the transport capabilities of the barrier under these conditions, as well as the effect of radiation, thermal energy, and chemotherapy will be studied. It is believed that an understanding of these changes, both in neoplastic and non-neoplastic pathological conditions, will allow experimental manipulation of the transformed blood-brain and blood-tumor barrier with subsequent interference with brain-tumor survival, the latter effect being due to alterations in the availability of metabolic substrates required for tumor metabolism.

BIBLIOGRAPHIC REFERENCES: Pollay, M. and Stevens, F.A. Simultaneous Measurement of Regional Blood Flow and Glucose Extraction in Rat Brain. Neurochemical Research 4:94-107, 1979. Pollay, M. and Stevens, F.A. Transport of Glucose into Rat Brain During Starvation. Journal Neurobiology, in press 1979.

398. STUDY OF EXPERIMENTAL CEREBRAL METASTASES AND THEIR VASCULATURE

Ballinger, W. E., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *Gainesville, Florida,* 32601, United States of America

The purpose of this study was to examine the cellular behavior and certain functional characteristics of cerebrovasculature in experimental cerebral metastases produced by injection of M3 fibrosarcoma cell suspensions into the carotid artery of C57BL/6 mice. This particular study utilized electron microscopic techniques in examining the arrest of tumor cells in the cerebrovasculature, their subsequent penetration into the perivascular space, and growth into metastatic tumor nodules. Also, horseradish peroxidase techniques were applied to determine the integrity of the blood-brain barrier within the cerebral vessels involved in this process. Autoradiography at the light and ultrastructural microscopic levels was used to determine rates of endothelial proliferation within these early metastatic foci.

The tumor cells were found in the cerebral capillaries from 15 minutes to 4 days following carotid injection. Tumor cells were first observed from 15 minutes to 18 hours post-injection to be surrounded by platelets and polymerized fibrin. From 24 hours on, tumor cells were also observed in the perivascular spaces between the basement membrane of the endothelial cells and the foot processes of the surrounding astrocytes. The tumor cells then proliferated in this space, displacing the cerebral tissue away from the vessels, and surrounded the vessels growing in a circumferential manner. Tumor cells were also seen growing logitudinally along these pre-existing cerebral vessels. We have shown that the cerebral endothelial cells are not permeable to horseradish peroxidase, even when surrounded by tumor cells up to 4 weeks of growth. The exact time at which the blood-brain barrier does break down in these metastatic nodules is still not answered; however, we are currently looking at animals that are in 5, 6, etc. weeks of tumor growth. Currently,several tumor bearing animals are being studied with 3H-thymidine to determine if endothelial cells do show proliferative activity which may be indicative of angiogenesis activity.

399. FINE STRUCTURE OF VIRAL INDUCED BRAIN TUMORS

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The blood-tumor capillary barrier to horseradish peroxidase permeability is being studied in three rat intracerebral glioma models (ASV, RG-2 and 9L), three rat subcutaneous glioma models (RG-2, 9L and S69) and human glioblastoma in nude mice. Dogs bearing ASV gliomas are being studied by CT and combined histological/histochemical methods. The goal is to characterize the differences between these models and to then use them appropriately for studies of the permeability, uptake and distribution of cancer chemotherapeutic agents. The cell kinetics of these models are being evaluated with four autoradiographic techniques: labeled mitoses, double labeling, continuous thymidine labeling and the stathmokinetic method. Tumor endothelium is being extracted for ultrastructural quantitation of fenestrations.

BIBLIOGRAPHIC REFERENCES: Groothuis, D.R., Fischer, J.M., Vick, N.A., and Bigner, D.D.: Experimental Gliomas: An autoradiographic study of the endothelial component. Neurology 30:297-301, 1980. Vick, N.A.: Brain Tumor Microvasculature in Weiss, L., Gilbert, H.A. and Posner, J., Brain Metastasis, G.K. Hall, 1980.

D. ROLE OF THE CELL MEMBRANE IN TUMOR GROWTH AND DIFFERENTIATION

400. REGULATION OF THE CELL CYCLE AND DIFFERENTIATION OF NEUROBLASTOMA CELLS IN VITRO - DYNAMIC PROPERTIES OF PLASMA MEMBRANE RECEPTOR SITES

van Zoelen, E. J., de Laat, S. W., van der Saag, P. T., Rijksuniversiteit te Utrecht, Hubrecht Lab., *Uppsalalaan 8, 3584 CT Utrecht, Netherlands*

OBJECTIVE: To reveal the molecular mechanism underlying the regulation of growth and differentiation of neuroblastoma cells, in particular the possible role of modulation in the expression of plasma membrane receptor sites.

APPROACH: The intrinsic modulations in the expression of a number of specific plasma membrane receptor sites is investigated. A possible relationship between the expression of receptor sites and their dynamic properties, measured as their lateral and rotational mobilities, is under study, with emphasis on the role of lipid-protein interactions.

401. REGULATION OF THE CELL CYCLE AND DIFFERENTIATION OF NEUROBLASTOMA CELLS IN VITRO - ROLE OF ION TRANSPORT

Boonstra, J., de Laat, S. W., van der Saag, P. T., Rijksuniversiteit te Utrecht, Hubrecht Lab., *Uppsalalaan 8, 3584 CT Utrecht, Netherlands*

OBJECTIVE: To reveal the molecular mechanisms underlying the regulation of growth and differentiation of neuroblastoma cells, in particular the possible role of the plasma membrane ion transport properties.

APPROACH: The intrinsic modulations in cation transport properties, including the plasma membrane-bound (Na plus-K plus)-ATPase, are studied by tracer-flux methods and biochemical techniques. The possible role of these membrane properties in the regulation of the cell cycle and differentiation is established directly by modification of the cation transport properties, and indirectly by experimental modification of the plasma membrane composition.

402. UPTAKE OF VARIOUS SUBSTANCES BY TUMORS

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OBJECTIVE: To reveal alteration of tumor cell membrane with reference to transport of substances.

APPROACH: 1. Uptake of nucleic acid precursors by transplantable tumors such as Yoshida ascites sarcoma, Walker 256 carcinosarcoma and AH7974 ascites hepatoma and by tumor bearing rat liver is investigated. 2. During diethylnitrosamine hepatocarcinogenesis, uptake of orotic acid and thymidine by rat liver is investigated. 3. The difference between uptake of nucleic acid precursors by mouse fibroblast A31 cells and by its transformant ASEA 7 cells (by SV40 virus) or between uptake by mouse neuroblastoma N-18 cells and by its MNNG-differentiated cells is investigated. 4. Changes in uptake of various substances in tumorous or normal tissue by compounds including chemotherapeutic agents are investigated.

403. MEMBRANE STRUCTURE AND ENZYME INDUCTION

Chen, K. Y., Rutgers, the State University of New Jersey, New Brunswick Campus, Douglass College, Dept. of Chemistry, *New Brunswick, New Jersey*, 08903, United States of America

The principal objective of this proposal is to study some of the biochemical processes that underlie differentiation; particular emphasis will be placed on the involvement of plasma membrane and ornithine decarboxylase (ODC). Mouse neuroblastoma cells can be induced to differentiate by cAMP. The differentiated cells lose their tumorigenicity. Increased tyrosine hydroxylase (TH) activity and neurite outgrowth are two distinct phenotypic expressions of differentiation in neuroblastoma cells. The possible involvement of membrane protein phosphorylation in mediating the effect of cAMP in cytodifferentiation will be studied. Differences in the composition of plasma membrane proteins of malignant and differentiated neuroblastoma cells will be probed by two surface labeling techniques (lactoperoxidase catalyzed iodination, and galactose oxidase catalyzed reductive tritiation), as well as by phosphorylation of purified membranes catalyzed by endogenous protein kinases. cAMP causes the induction of ODC and TH in neuroblastoma cells. These two enzymes are the rate-limiting enzymes in the synthesis of polyamines and catecholamines, respectively. The mechanism by which cAMP induces ODC, and the involvement of ODC in cytodifferentiation will be studied. The approach is to delineate the relationship between cAMP-induced membrane protein phsophorylation changes and the increase in ODC activity. The temporal and/or causal relationship between the cAMP-induced increase in ODC activity and TH activity will be investigated. The approach is to correlate changes of the two enzyme activities under conditions where ODC activity is varied. The proposed studies should help us to understand the role of cAMP and polyamines in cytodifferentiation. In addition, knowledge on the composition of plasma membranes of malignant vs. differentiated cells should help us to gain a better insight into the understanding of tumor reversion. Our long-term goal is to be able to design drugs which specifically modulate the various biochemical processes which underlie differentiation, so that differentiation and tumor reversion can be manipulated pharmacologically.

404. BIOCHEMISTRY OF INOSITOL AND PHOSPHOINOSITIDES

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The overall purpose of this research is the elucidation of all aspects of phosphoinositide metabolism, their involvement in the detailed organization and functioning of membranous structures and their participation in physiological phenomena. Their high rate of renewal is indicative of a fundamental role in cell membranes which these studies are designed to clarify.

During the present project period one major goal is to study further the mechanism and significance of the stimulation by external agents of the turnover of phosphatidylinositol and other acid phospholipids in vitro, the phospholipid effect. The nature and characteristics of alpha-adrenergic receptors mediating this effect in rat pineal gland and other suitable tissues including neuroblastoma cell cultures and their close coupling to phospholipid metabolism are to be determined. The mechanism by which norepinephrine and other agonists exert their effects will be studied through the measurement of changes in the level of individual phospholipids and in the extent of incorporation of radioactive precursors. Alterations in incubation conditions, pulse chase experiments, assays of individual reactions of phospholipid metabolism and experiments with plasma membrane and other subcellular fractions will be used to elucidate the events occurring in stimulated tissues. Fatty acid patterns of phospholipid classes will be examined and changes in calcium fluxes resulting from the increased phospholipid turnover will be measured.

The other major goal is the elucidation of the redirection of phospholipid metabolism by cationic amphiphilic drugs, such as propranolol and psychoactive agents. The spectrum of drugs capable of causing this effect in vitro, possibly through inhibition of phosphatidate phosphohydrolase, will be determined and compared with those able to cause human phospholipidosis in chronic use. The mechanism of the phenomenon is to be explored, especially in relation to calcium ions and charges on cell membrane constituents. The role of inositol in influencing and controlling metabolic processes and lipid interconversions will also be analyzed.

405. MEMBRANE BIOCHEMISTRY IN DRUG RESISTANT NEUROBLASTOMA

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Neuroblastoma, treated with a variety of chemotherapeutic drugs, frequently responds, but drug-resistant tumors generally return. To explore these drug resistance mechanisms we isolated and biochemically characterized mutant clones of a spontaneous murine C-1300 neuroblastoma in monolayer cell culture resistant to antifolates, anthracyclines and microtubule disrupters. Maytansine-vincristine-adriamycin-Baker's antifol reciprocally cross-resistant cells, resistant to structurally and functionally diverse drugs, are unable to accumulate these drugs despite normal binding to cell-free homogenates. This lowered tumorigenicty in the syngeneic Ajax mouse and collateral sensitivity to glysosylation inhibitors led us to examine the resistant cell's plasma membrane glycoproteins and glycolipids using whole cell metabolic or surface radiolabeling techniques and fractionation by 2-dimensional gel electrophoresis or thin layer chromatography. All mutants demonstrate specific but pleiotropic changes in cell-surface glycoproteins and gangliosides suggesting a common glycoconjugate altering enzyme. Double-minute chromosomes, seen in unstable but not more highly resistant stable drug-resis-

tant descendents, may contain amplified gene copies of the resistance mediating protein. One glycoprotein - X, MW 100,000, pI equals 4.0 - missing from several mutants and present in a revertant, may be causal. We propose to determine whether this or other altered glycoconjugates represent a glycosyl-transferase, glycosidase, protein synthesis or membrane assembly defect, to contrast this mechanism with that of diploid human neuroblastoma SK-SY-5Y drug-resistant clones, and to compare the effect of the membrane glycosylation inhibitor, tunicamycin, on drug-resistance and altered tumorigenesis in an effort to better understand and improve tumor chemotherapy.

BIBLIOGRAPHIC REFERENCES: Prashad N, Rosenberg R, Baskin F, Sparkman D, Ulrich C and Wischmeyer B. Cyclic adenosine 3'5' monophosphate-binding protein, a biochemical marker of neuroblastoma differentiation. Cancer Research, 40, 2884-2889, 1980.

406. ANTIOXIDANTS IN MOUSE NEUROBLASTOMA

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The membranes of mouse neuroblastoma cells undergo a dramatic increase in resistance to lipid peroxidation during differentiation. A sensitive, single-phase assay for antioxidant activity was developed in this laboratory in order to test the hypothesis that a lipid-soluble antioxidant was generated by these cells. Studies on lipid extracts revealed several antioxidant fractions but none of these increased in potency during neuroblastoma differentiation. Thus, the increased membrane resistance did not appear to be due to an antioxidant. In addition, studies on the lipid composition of differentiated and undifferentiated neuroblastoma cells have failed to reveal changes which could explain the increase in resistance. The hypotheses that differences in membrane structure can account for the increased resistance is presently under investigation. Particularly interesting is the possibility that differentiated membranes might exhibit resistance to lipid peroxidation after detergent-solubilization and reconstitution.

407. ROLE OF GANGLIOSIDES IN THE PATHOGENESIS OF GLIOMAS

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The long-range goal of this project is to understand at the molecular level the pathogenetic mechanisms responsible for the uncontrolled proliferation of human gliomas. Current evidence indicates that the cell surface plays a critical role in normal cellular growth control. Gangliosides are a class of glycolipids located at the cell surface which probably participate in several biological functions one of which may be the control of cell division. Ganglioside compositions different from parent tissues have been found in many tumor types, including gliomas, which may be related to their lack of cellular growth control. Gangliosides appear to be shed from the surface of glioma cells into the blood and evidence from our laboratories and others indicates that gangliosides suppress the immunoreactivity of peripheral blood mononuclear cells. Thus, it is possible that these shed gangliosides may be responsible for immunodeficiencies in patients with gliomas. In this proposal three groups of experiments have been designed to investigate the roles of gangliosides in phenomena relevant to the pathogenesis of human gliomas.

In the first group we will study aspects of ganglioside metabolism in cultures of human glioma and normal glial cells under different growth conditions to determine if their ganglioside metabolism is related to cell density and/or cell multiplication. Comparisons between normal and neoplastic glia will be made.

The second group includes experiments to investigate the effects of exogenously added gangliosides on several biological functions relevant to cell multiplication of normal and neoplastic cultured glial cells.

The third group of experiments has been designed to investigate whether or not gangliosides in human gliomas and serum of patients with gliomas are capable of suppressing the reactivity of human normal peripheral blood mononuclear cells to non-specific mitogens.

408. LIPID INTERMEDIATES IN MEMBRANE PROTEIN GLYCOSYLATION

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There is now considerable information on changes in the nature of oligosaccharide units linked to cell surface glycoproteins. In order to understand how these alterations occur, and what the relationship is to malignant transformation, it is necessary to know precisely how these carbohydrate units are assembled.

The goal of this project is to elucidate the biosynthesis, structure and function of polyisoprenoid glycolipids in the central nervous system. The structure and identity of the membrane glycoproteins glycosylated via lipid intermediates will be investigated. Determining if the cellular level of dolichyl monophosphate, the lipophilic glycosyl carrier, plays a role in regulating the rate of glycoprotein assembly will be another important goal. Since it is possible that the membrane content of dolichyl monophosphate could influence the rate of glycosylation of membrane glycoproteins, we are also going to attempt to detect dolichol kinase and dolichyl pyrophosphate phosphatase activity in brain membranes. Both of these enzymes would serve important functions in the metabolism of dolichyl monophosphate.

This project also includes enzymatic studies on the biosynthesis of dolichol-linked sugars by membrane preparations from brains of 'Jumpy' and 'Quaking' mice, two neurological mutants deficient in CNS myelin.

409. GLYCOLIPID METABOLISM IN TUMOR AND TRANSFORMED CELLS

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The major objectives of the proposed research are twofold: 1) Isolation, characterization and biosynthesis of glycosphingolipids of the globoside family in guinea pig embryonic 103 cells and their chemically transformed variants 104Cl(benzo(a)pyrene), 106B(7,12-dimethylbenz(a)anthracene and 107C3 (N-methyl-N'-nitro-N-nitrosoguanidine). Differential binding of 125 I-labeled lectins (Dolichos biflorus, Bandeiraea simplicifolia and toxins (cholera and ricin) to these cell surfaces under various growth conditions.

2) Studies on the structure and biosynthesis of N-acetylglucosamine-containing blood group related glycosphingolipids (sialo- and fuco-) in human neuroblastoma IMR-32 cells, mouse neuroblastoma N-18 and mouse T- and B-lymphoma cells. Differential binding of 125I-labeled lectins (Ulex europeus, Euonymus europeus and Lotus tetragonolobus) and toxins (cholera and ricin) to N-18 and IMR-32 cell surfaces before and after chemically induced (6-MG, BrdUrd and (But)2cAMP) differentiation.

410. HISTOPATHOLOGIC STUDY OF PIGMENTED OCULAR TUMORS

Albert, D. M., Massachusetts Eye & Ear Infirmary, *243 Charles St., Boston, Massachusetts,* 02114, United States of America

The present proposal is for the continued investigation of the etiology, pathogenesis, and pathobiology of ocular melanomas and retinoblastomas. It will utilize observations made and techniques developed in earlier research supported by this grant. In addition, new methods of investigation will be employed. The following objectives are proposed for the next five years: 1. Morphological studies employing parallel light microscopy, transmission electron microscopy, scanning electron microscopy, and freeze-fracture techniques, as appropriate, to examine in detail the cell surface, intrinsic membrane structure, microtubular and fibrillar structure, cell junctions, and other ultrastructural details. These structures will be compared for the normal, 'premalignant', dysplastic and various classifications of malignant cells for uveal malignant melanoma and retinoblastoma. Additional morphologic studies of inflammatory cells in malignant melanoma, sequence of degeneration of retinoblastoma and karyotypic studies in retinoblastoma and melanoma. 2. Molecular biology studies regarding the control of melanin synthesis in malignant and benign ocular pigmented cells, retinoid binding sites on retinoblastoma and melanoma cells, and radiosensitivity studies related to DNA-repair defects in retinoblastoma and

melanoma patients. 3. Development of tumor models focusing on melanoma in the nude mouse, in addition to established melanoma and retinoblastoma cell lines; malignant transformation of ocular tissues with herpes simplex virus and nickel subsulfide. 4. Application of tumor models to clinical questions, including the effect of enucleation on tumor cell dissemination and metastasis; the usefulness of adjunct radiotherapy; and retinoids in the treatment of retinoblastoma.

411. ROLE OF MATRIX AND CELL SURFACE IN NEURAL CREST CELL MORPHOGENESIS

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Neural crest cells migrate extensively during development. This invasive behavior is spatially and temporally precise, and the cells eventually form such diverse derivatives as: pigment cells, sensory and autonomic ganglia, teeth and the cranial connective tissues. Changes in distribution and composition of the extracellular matrix and the presence or absence of cell associated proteins on the crest cells may be related to the onset of neural crest cell movement and to the control of the pattern and pathway of their migration.

There are a number of mutant mouse strains which are deficient in neural crest cell migration and differentiation and express such abnormalities as loss of pigmentation, cranial-facial lesions and absence of sensory and autonomic ganglia.

I plan to test the role of the extracellular matrix and cell surface proteins in the control of neural crest cell migration by 1) correlating these factors with normal crest morphogenetic events and 2) comparing changes in these factors with concomitant morphogenetic events in mutant mice.

412. BIOLOGICAL FUNCTIONS OF PLASMA MEMBRANE SIALIDASE

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In this study cell surface changes associated with the in vitro growth and development of dissociated embryonic cerebrum cells, and the maturation of cultured oncogenic, transformed nervous system cells will be determined. More specifically studies will be continued to 1) try to improve upon the purity of the neurons grown in vitro after centrifugal elutriation of dissociated embryonic rat cerebra; and 2) to elucidate some of the cell surface changes in neuroblastoma cells as they change from the pre-confluent to the post-confluent stage of growth in vitro. In addition in vivo studies designed to determine why neonatal mice are less susceptible than adult mice to neuroblastoma development upon challenge with neuroblastoma cells will be continued.

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413. TUMOR SPECIFIC CELL MEMBRANE MARKERS

Sliski, A. H., Todaro, G. J., Sherwin, S. A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Viral Leukemia & Lymphoma Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this work is to study tumor-specific cell membrane markers and elucidate their role in the process of transformation. Human tumor cells have been shown to produce a variety of growth factors. We concentrated on nerve growth factor (NGF) and demonstrated that cells derived from the neural crest when transformed both produce and bind nerve growth factor. This may be an example of 'autostimulation' by tumor cells which allows them to proliferate independent of exogenous growth control. A membrane immunofluorescence test was developed which is able to detect NGF receptors and NGF production on human melanoma and glioblastoma cell lines. Tests done on fresh human tumors derived from the neural crest provide the same results. A variety of human sarcoma, carcinoma and normal cell lines did not show NGF binding or production. NGF and its receptor may be the melanoma tumor antigen known to cause an immune response in patients. This assay may prove useful for monitoring the clinical course of a patient.

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414. SCHWANN CELL MEMBRANE AND SCHWANN CELL-AXOLEMMA INTERACTIONS - A MORPHOLOGICAL STUDY OF THE GARFISH OLFACTORY NERVE (ABBREV)

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Schwann cell membranes from the myelin sheaths of rat optic and sciatic nerves are observed during differentiation. Freeze-fracture analysis is used to determine the processes of myelin formation. Hexagonal particle rosettes are observed at the periaxonal regions of the Schwann cell plasma membrane in regional correspondence with particle clusters in the axolemma.

415. RETROGRADE MEMBRANE MOVEMENT ON SYMPATHETIC NEURITES

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Retrograde movement of a number of membrane markers along the neurites of embryonic chick sympathetic neurons has recently been demonstrated. The overall objective of this project is to characterize further these membrane movements and to evaluate their physiological significance. The following experimental approaches will be utilized: (1) the mechanics of retrograde marker movement will be examined in detail using a video camera and time-lapse recorder, (2) the embryonic retinotectal fiber system will be used as a model to determine whether this process also occurs in vivo, (3) other types of neurons in primary culture and neuroblastoma cells will be examined to determine whether retrograde membrane movement occurs on all types of neurons, (4) neuronal and non-neuronal perikarya from sources other than sympathetic ganglia will be added to sympathetic cultures to determine whether these cell bodies will also attach and be transported back along the neurites, (5) membrane specialization at the sites of attachment of membrane markers to the axolemma will be sought by use of electron microscopy, (6) this process will be characterized pharmacologically using drugs known to affect specific metabolic processes, and (7) a hypothetical model for the mechanism of addition of new membrane elements to growing and static nerve fibers will be tested.

416. PHOSPHOLIPID METHYLATION AND MEMBRANE STRUCTURE AND FUNCTION

Hirata, F., Strittmatter, W. J., Axelrod, J., Crews, F., Tallman, J., Schiffmann, E., U.S. Dept. of Health & Human Services, Public Health Service, Alcohol, Drug Abuse & Mental Health Admin., National Inst. of Mental Health, Section on Pharmacology, *Bethesda, Maryland*, 20014, United States of America

Beta-adrenergic agonists stimulate phospholipid methylation in HeLa cells and astrocytomas. Benzodiazapam acid anti-anxiety drugs also stimulate phospholipid methylation in astrocytoma cells. Ca(plus plus) ATPase activity is increased after synthesis of phosphatidylcholine is elevated. An association between histamine release in mast cells and phosphatidylserine decarboxylation and phospholipid methylation was observed. Also a close relationship between lymphocyte mitogenesis, phospholipid methylation and arachidonic liberation was found. Chemotactic peptides caused an increased turnover of methylated phospholipids and release of arachidonic acid. Two phospholipid methylating enzymes were found in brain nerve endings.

BIBLIOGRAPHIC REFERENCES: Hirata, F., Axelrod, J. and Strittmatter, W.J.: Methylation of membrane phospholipids. In Usdin, E., Borchardt, R. and Creveling, C. (Eds.) Transmethylation, Vol. 5. New York, Elsevier/Holland Press, 1979, pp. 233-240. Hirata, F., Strittmatter, W.J. and Axelrod, J.: Beta-adrenergic receptor agonists increase phospholipid methy-

lation, membrane fluidity and beta-adrenergic receptor-adenylate cyclase coupling. Proc. Natl. Acad. Sci., USA, 76: 368-372, 1979.

417. SODIUM-DEPENDENT HIGH AFFINITY TRANSPORT IN GLIA

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The long-term goal of the proposed research is to understand the mechanisms by which glia cells help to regulate the extracellular environment in the brain. The premise underlying this work is that high-affinity membrane transport systems in glia are important contributors to the regulation of the extracellular environment and that a more complete understanding of the mechanism of these systems is needed to evaluate their contributions. All high-affinity systems studied so far are Na(plus)-dependent. The energetics of sodium-dependent systems is thought to depend on Na(plus) and K(plus) ion gradients across the membrane, the membrane potential, transport stoichiometry, and the electrical charge on the substrate. We plan to investigate the transport of three neurochemically important compounds, taurine, glutamate, and choline, by rat glioma cells maintained in cell culture. These studies are designed to determine whether the ion gradients and membrane potential do, in fact, provide the energy for the transport of these three compounds and the extent to which changes in membrane potential can influence the rate and direction of transport.

418. PLASMA MEMBRANE OF NEUROBLASTOMA CELLS (MICE, ELECTRIC EEL)

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This is part of a broader project. A summary of this subproject is not available.

E. GENE EXPRESSION IN NERVOUS SYSTEM TUMORS

419. BIOSYNTHESIS OF A BRAIN-SPECIFIC S100 PROTEIN IN A CLONAL LINE OF RAT GLIAL CELLS

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The relative synthesis of the brain-specific S100 protein increases in clonal rat glial cells C6 when the cells progress from logarithmic to stationary growth in monolayer culture. The induction of S100 protein is paralleled by an accumulation of its specific mRNA. Intercellular contact has been postulated to be the stimulus for increased synthesis of S100 protein. We have attempted to define by biochemical and genetic approaches the steps in the pathway involved in S100 protein induction; specifically, extracellular signals trigger cell surface changes which cause intracellular mediators to trigger molecular mechanisms of protein synthesis.

The lectin succinylated concanavalin A induces the synthesis of S100 protein in logarithmic cultures of C6 cells. Drugs which disrupt microtubules inhibit the synthesis of S100 protein in stationary cultures of C6 cells. Colchicine inhibits the accumulation of S100 protein mRNA.

We propose to assess genetically the role of succinyl Con A, cyclic AMP and microtubules in S100 protein induction. Induction of S100 protein will be studied in C6 cell mutant clones resistant to colcemid, concanavalin A and 8-BrcAMP and attempts will be made to link biochemically the genetic lesions to S100 protein synthesis. The influence of microtubules on nuclear transcription, processing and transport of mRNA will be studied using a cDNA probe complementary to S100 protein mRNA. The demonstration of a lectin-glycoprotein recognition system on the surface of C6 cells will be attempted.

BIBLIOGRAPHIC REFERENCES: Marks, A., Mahony, J.B. and Brown, I.R. (1978) Colchicine inhibits the accumulation of messenger RNA for a brain-specific protein in rat glial cells. Biochem. Biophys. Res. Commun. 82, 1306-1313.

420. PROGRAM IN DEVELOPMENTAL BIOLOGY OF CANCER

Pierce, G. B., Erikson, R. L., Lehman, J. M., Nakane, P. K., University of Colorado, Health Sciences Center, School of Medicine, Dept. of Pathology, *4200 E. 9th Ave., Denver, Colorado*, 80262, United States of America

It has been shown through the study of three kinds of carcinoma that the progeny of malignant stem cells have the capacity for differentiation, yielding benign and, in one case, normal progeny. Direction of this naturally occurring differentiation of tumors could serve as an alternative to cytotoxic therapy. To this end, a multidisciplinary approach to the study of differentiation in tumors will be mounted. It will include input from experimental pathologists, cell biologists, molecular biologists, biochemists, and developmental biologists. Focus will be on three essential processes: the environment of differentiating cells, the plasma cell membrane which must mediate environmental signals, and finally, the way neoplastic genomes are controlled.

Specifically, we will study control of genomes through the study of the synthesis of the transforming protein encoded for in the src region of RNA tumor viruses. Membrane studies will include molecular studies of transbridging proteins and comparisons of the metabolisms of normal and neoplastic membranes including the distribution and turnover of glycoproteins, and effects of growth promoters on membranes. Comparisons will be made of asymmetrical distribution of proteins of membranes of karyoplasts and nucleoplasts of normal and malignant cells to obtain information on cell-cell interactions.

Comparison will be made of the effects of DNA tumor viruses on differentiation of cells or early embryos and of teratocarcinomas. Flow microfluorimetry will be done on temperature-sensitive mutants of SV40 to determine the mechanism of action. The role of embryonic fields in directing differentiation of neoplastic cells will be studied. Functional differentiation in developing pituitary glands, pituitary tumors, and in liver cell will be studied.

BIBLIOGRAPHIC REFERENCES: Miller, G.J. and Pierce, G.B. Induction of foam cell morphology in aortic media cells by phorbol-12-myristate-13-acetate (PMA). Fed. Proc. 39:429, 1980. Pierce, G.B. Teratocarcinoma. In: Cancer Markers: Developmental and Diagnostic Significance (Stewart Sell ed.), Humana Press, Inc., Clifton, N.J., 1980, pp. 1-36.

421. PROGRAM PROJECT IN CLINICAL GENETICS

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Work in the Wisconsin Clinical Genetics Center (WCGC) includes: 1) Delineation of new birth defect syndromes, their cause, natural history, prognosis and treatment; 2) study of 'known' birth defects to better delineate their phenotypic spectrum, pathogenesis and cause, and the means to detect carriers and to detect affected individuals prenatally; 3) cytogenetic studies of new and known aneuploidy syndromes; 4) studies of genetic pleiotropy in man; 5) investigations of the nature of gene expression in tissues, organs and cells of individuals (expressivity), in individuals vis-a-vis their genotype (penetrance) and in generations vs. their genotypes (phenotrance), in skeletal dysplasias (achondroplasia, hypochondroplasia), malformation syndromes (Wiedemann-Beckwith syndrome, prune belly syndrome, etc.), and cancers (retinoblastoma, Wilms' tumor, carotid body tumors, multiple glomus tumors and multiple cutaneous leiomyomata; 6) studies of methods of numerical taxonomy in clinical studies and genetic nosology; 7) studies of prenatal diagnosis, the incidence of Down's syndrome and neural tube defects in Wisconsin; 8) studies of the prevalence of developmental disabilities in Burnett Co. and Dane Co. Wis.; 9) health and genealogical surveys of all of the Old Order Amish in Wisconsin, Illinois, Iowa, Missouri and Minnesota; 10) total dissections of fetuses and infants with known (genetic) syndromes and experimental studies of pathogenesis of congenital malformations in aneuploid mice; 11) causal, nosological and empiric recurrence risk studies in severe MR -especially that associated with primary CNS malformations; 12) mutational and genetic analysis of the experimentally (EMS) induced phenomenon of unstable pink mutation in Drosophila; 13) interdisciplinary studies of phenotype, pathogenesis, cause, nosology and recurrence risk in skel-

etal dysplasias and human intersexuality; 14) studies of the effects on offspring of parental consanguinity in Wis. rural Catholics; 15) studies on the pathology, anatomy and genetic defects of aborted fetuses and stillborn infants; 16) studies of mucopolysaccharide metabolism and of lysosomal hydrolases in human mucopolysaccharidoses, mucolipidoses, and lipidoses; 17) biochemical, EM, and histochemical studies of many pigment mutations in mouse, mink, and man.

BIBLIOGRAPHIC REFERENCES: Marcallo, F.A., Werneck, L.C., Pilotto, R.F., Opitz, J.M. Hemihypotrophy in a girl with a translocation t(13q;7p). Europ. J. Pediat. 124:167-171, 1977. Neuhauser, G., ZuRhein, G.M., Kaveggia, E.G., Opitz, J.M. Fatal CNS dysgenesis with severe microencephaly, mental retardation, seizures and paucity of myelin; autosomal recessive trait. Europ. J. Pediat. 124:185-198, 1977.

422. COMPLEXITY OF GENE EXPRESSION - CLONING

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We propose to establish DNA clones whose RNA transcripts are found exclusively in the brain cytoplasm (brain-specific) and use them to strengthen our overall research project on the gene expression in the nervous system. The overall objective of this research is to understand why brain transcripts have an unusually large complexity compared with those of other tissues. For this, we study the transcriptional complexity (the fraction of rat genome expressed as RNA) of various rat tissues, tumors, and their derivative cell lines, with special emphasis on the nervous system. In order to make our research more incisive, we propose to isolate DNA clones which represent a portion of the genome specifically transcribed into messenger RNAs (mRNA) of the brain. DNA fragments of the entire rat genome have been amplified in a bacteriophage lambda, from which we will select those for brain mRNAs. We expect to isolate several classes of brain-specific DNA clones with different quantitative levels of transcription, i.e., rare, abundant, and intermediately abundant classes. These clones will be extremely useful in furthering our studies of gene expression in the brain and other organs.

BIBLIOGRAPHIC REFERENCES: Sueoka, N., D.M. Chikaraishi, S.S. Deeb, P. Hseih, M. Imada and Y. Tomozawa (1979). Cell type conversion of a rat neural stem cell line in vitro and complexity of gene expression in rat brain. Proc. XIV Internat. Cong. Genetics, 1978, Moscow, in press.

423. INTERDISCIPLINARY RESEARCH PROGRAM IN HUMAN CELL BIOLOGY - GENETIC ASPECTS OF HUMAN CELL REGULATION

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This is a collaborative project in human biology involving 5 independent laboratories. Each project is examining in a different way the genetics of human cell differentiation. The individual projects are as follows: (1) Alkaline phosphatases of man as expressed in tissue culture; (2) the reversion of missense mutations at the human gal-1-p-uridyl transferase locus using SV40 transformed cells from mutant patients; (3) the genetic control of neural functions in cell lines derived from neuroblastomas; (4) the genetic control of the expression of human differentiation antigens; and (5) the expression of human differentiated functions in hybrids between teratocarcinoma cells and mouse cells with reduced numbers of human chromosomes implanted into mosaic mice. These are highly interactive projects and are dependent not only on the expertise of the collaborating individuals but also on the exchange of cultured human cell lines and hybrid cell lines between human and rodent cells.

424. CELL BIOLOGY EXAMINING HUMAN GENE REGULATION

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This is a coherent area grant involving four laboratories concerned with genetics of cell regulation and differentiation. Project I uses monoclonal antibodies to identify and isolate human cell surface differentiation antigens. A 'library' of monoclonal antibodies against antigens on a collection of different human cell types is being produced. These are to be used to define cell suface changes that take place during cellular differentiation. Project II is directed toward an understanding of the structure and control of the genes for human collagen in normal and SV40 transformed human fibroblasts. This provides a way to study the differential expression of the three structural genes for collagen polypeptides. Project III uses cloned lines derived from human neuroblastomas to study the genetic regulation of the neural phenotype. These cells are to be characterized with regard to the expression of neurotransmitter enzymes and catecholamines. Project IV involves analysis of the membrane components of human neuroblastoma and normal human neurons using monoclonal antibodies to compare the cell surface structure of neuroblastomas and normal neural tissue.

425. BIOCHEMICAL GENETICS OF OVARIAN AND TESTICULAR TERATOMAS

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It has been found that cultured neuroblastoma and teratoma cells co-aggregate with cerebellar cells from postnatal mice. Cerebellar cells aggregated by themselves differentiate in vivo both morphologically and biochemically. We have studied the transition from embryonic L-glycerol-3-phosphate dehydrogenase (alpha-GPDH) isozyme to the adult-type expression in these cerebellar aggregates and have found that if cerebellar cells are co-aggregated with tumor cells then isozyme transition is inhibited. The cerebellar cells continue to synthesize embryonic isozyme rather than switch to adult isozyme expression. Several types of experimentation suggest that cell-to-cell interaction, possibly mediated through the plasma membrane or cell surface, is involved in the repression of the adult-type isozyme. We propose to investigate the mechanism by which this repression occurs by analyzing cerebellar cell aggregates that have been co-aggregated with disrupted cell preparations and purified subcellular fractions. If a subcellular fraction is identified then further experimentation will include isolation and identification of the chemical structures involved in these interactions. Other experiments will include immunofluorescence and ultrastructural analysis of co-aggregated cultures in order to assess whether there is a failure of cell sorting or cell-to-cell contact in the aggregates.

426. (CORE) PATHOGENESIS OF NEUROFIBROMATOSIS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

F. ENZYME REGULATION IN NERVOUS SYSTEM TUMORS

427. TYROSINE HYDROXYLASE - MECHANISMS OF REGULATION

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The enzyme tyrosine hydroxylase catalyzes the rate-limiting step in the biosynthesis of catecholamine neurotransmitters. The major aim of the experiments described in this proposal is to characterize the following three biochemical events which rapidly modify tyrosine hydroxylase activity by changing the affinity of the enzyme for its pterin cofactor: 1) anionic phospholipids, 2) enzymatic phosphorylating conditions, and 3) partial proteolysis. Each of these events can activate tyrosine hydroxylase; a comparative study will yield information concerning whether or not the activations occur via a common or through distinct mechanisms.

One class of anionic phospholipids, the phosphoinositides, have the remarkable ability to either stimulate or inhibit tyrosine hydroxylase, depending upon preincubation conditions. The phosphoinositides comprise a very small portion of the total phospholipids in nervous tissue, but they are the only class of phospholipids which turn over rapidly in response to cellular

stimuli, e.g., depolarization. Experiments have been designed to characterize the mechanism(s) of how the phosphoinositides may modulate tyrosine hydroxylase activity, using both purified enzyme preparations and synaptosomal or neuroblastoma cell culture preparations where in situ tyrosine hydroxylation may be measured.

In the course of the purification of tyrosine hydroxylase, a factor has been isolated which prevents the phosphatidylinositol-induced inactivation of this enzyme. Experiments have been designed to isolate and characterize this potentially physiologically important endogenous modulator of tyrosine hydroxylase activity.

BIBLIOGRAPHIC REFERENCES: Lloyd, T., Montgomery, P. and Guroff, G.: The receptor-mediated, short-term activation of tyrosine hydroxylase in organ cultures of rat superior cervical ganglia. Biochem. Biophys. Res. Commun. 86:1058-1062, 1979. Lloyd, T.: The effects of phosphatidylinositol on tyrosine hydroxylase: stimulation and inactivation. J. Biol. Chem. 254:7247-7254, 1979.

428. SYNTHESIS, STORAGE AND RELEASE OF CATECHOLAMINES

Weiner, N., Nakane, P. K., Murphy, R. C., Lee, F., Mosimann, W. F., Dixon, W., Stephens, J. K., Takimoto, G. S., Masserano, J. M., Tank, A. W., University of Colorado, Health Sciences Center, School of Medicine, Dept. of Pharmacology, *4200 E. 9th Ave., Denver, Colorado,* 80262, United States of America

Tyrosine hydroxylase from rat pheochromocytoma will be purified to homogeneity and a monospecific antiserum to the enzyme will be produced in rabbits. The enzyme from rat adrenal and striatum, guinea-pig vas deferens and mouse neuroblastoma cells will also be purified and the physical and kinetic characteristics of each enzyme and the changes in the kinetic properties of each enzyme produced by the cyclic AMP-dependent protein phosphorylating system, phosphatidyl-L-serine, cations and other putative regulators of the enzyme will be compared. The molecular basis of the physiological activation of tyrosine hydroxylase consequent to nerve stimulation will be explored, with particular attention directed to enzyme phosphorylation, or phosphorylation of an activator protein, and the role of calcium. Protein kinases and phosphoprotein phosphatases from each tissue will be purified and characterized and their possible relevance to the activation and deactivation of tyrosine hydroxylase will be determined. The mechanism of induction of tyrosine hydroxylase in the adrenal of rats and in mouse neuroblastoma cells in culture will be investigated further, and the role of protein kinase and cyclic AMP in this process will be ascertained. The ontogenic development of sensitivity to putative allosteric modulators of tyrosine hydroxylase will be defined in striatum of rat fetuses and young animals. The ultrastructural localization of tyrosine hydroxylase will be determined by immunocytochemical techniques and changes in the ultrastructural distribution of the enzyme with nerve stimulation will be examined. Regulation of catecholamine release consequent to nerve stimulation from isolated, perfused spleens will be evaluated with particular emphasis on cyclic nucleotides, alpha and beta adrenoceptor, dopamine and cholinergic agonists and adenosine. The uptake of tyrosine into adrenergic neurons and the equilibration of precursor amino acids in this structure will be assessed. Amino acid aminotransferases and keto acids will be evaluated with regard to their effects on maintenance of available neurotransmitter stores during prolonged nerve stimulation.

429. REGULATION OF GLYCOLYTIC ENZYMES IN DIFFERENTIATED NEUROBLASTOMA CELLS

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Lactate dehydrogenase (LDH) activity in neuroblastoma cells treated with several differentiating agents has been determined. In addition, the regulation of pyruvate kinase (converts phosphoenolpyruvate to pyruvate) in neuroblastoma cells was also investigated. Since both LDH and pyruvate kinase (PK) are involved in glycolytic pathway, we have investigated the changes in activities of these enzymes in 'differentiated' neuroblastoma cells induced by different agents.

We found that: (a) Sodium butyrate when administered before or after X-irradiation increases the lethal effect of radiation on mouse NB cells in culture; (b) sodium butyrate inhibits the activities of LDH and PK in non-irradiated neuroblastoma cells, whereas X-irradiation increases them; and (c) cyclic AMP stimulating agents elevate the LDH activity, but they prevent the ethyl alcohol-induced PK activity.

The presence of anoxic cells in tumor constitutes a major problem in radiation therapy because they are resistant to X-irradiation. Anoxic cells presumably use anaerobic glycolysis as a major source of energy. Therefore, it is possible that sodium butyrate may inhibit anaerobic glycolysis in these cells and thereby increase the radiation response of anoxic cells. If the present findings can be applicable in vivo, the administration of sodium butyrate after irradiation may improve the effectiveness of radiation therapy in human neuroblastomas as well as in other neoplasms. Further exploration of these experiments is obviously needed.

Progress has been good. The following publications were either made or are being prepared for publication: (1) Effect of cyclic-AMP stimulating agents on glucose-6-phosphate dehydrogenase in neuroblastoma cells. XV Inter. Congress of Pediatrics, 1977, p. 220. (2) Neuroblastoma cells: Effects of sodium butyrate, X-irradiation and cyclic AMP. Radiology (in press). (3) Regulation of lactate dehydrogenase and pyrurate kinase in mouse (In preparation).

430. REGULATION OF ENZYME ACTIVITY IN NORMAL AND TUMOR CELLS

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Ornithine decarboxylase ((ODC) EC 4.1.1.17) catalzyes the synthesis of putrescine; this serves as the common diamine component of spermidine and spermine. ODC is present in all cell types and induction of ODC activity occurs under a variety of conditions associated with growth. We have shown that neuroblastoma, L1210, P388, H-35 cells, as well as 3T3 fibroblasts in culture and rat liver cells have available to them a number of mechanisms to regulate ODC activity in a very sensitive manner.

The research of our laboratory has defined a unique mechanism of control of ODC activity. The end products of the reaction elicit the appearance of a specific small molecular weight protein, ODC antizyme, which is a non-competitive inhibitor of ODC and forms a salt-dissociable complex with ODC. It is proposed: (a) To purify and characterize the ODC antizyme and its mode of regulation of ODC activity; (b) to define the intracellular complexes that it forms with ODC; (c) to define how the antizyme interacts with other factors that are co-induced in the cell; (d) to determine the minimal requirements, as well as the discrete steps involved in the induction of ODC and of the ODC antizyme.

431. REGULATION OF BRAIN AND TUMOR PHOSPHOTRANSFERASE

Fromm, H. J., Solheim, L., Stayton, M., Shone, C., Stone, S., Iowa State University of Science & Technology, School of Agriculture, Dept. of Biochemistry & Biophysics, *Beardshear Hall, Ames, Iowa,* 50010, United States of America

Studies will be undertaken to study the kinetic mechanism of the fructose-1,6-bisphosphatase reaction using substrate analogs such as 2,5-anhydromannitol-1,6-bisphosphate, phosphite, and 2-deoxyribose-5-P. Phosphite and 2-deoxyribose-5-P are competitive inhibitors of phosphate and fructose-6-P, respectively, when the reverse reaction is studied. We will determine the type of inhibition exhibited by these analogs relative to the substrate that is not competitively inhibited. These experiments should provide information on the enzyme's kinetic mechanism. In addition, studies of both the forward and reverse reaction with 2,5-anhydromannitol-1,6-bisphosphate should provide us with additional information on how fructose-1,6-bisphosphatase functions as a catalyst. We also plan to study the regulation of the enzyme by the allosteric effector AMP.

BIBLIOGRAPHIC REFERENCES: Stayton, M. M., and Fromm, H. J. (1979) Guanosine-5'-diphosphate-3'-diphosphate Inhibition of Adenylosuccinate Synthetase. J. Biol. Chem., 254, 2579. Stayton, M. M., and Fromm, H. J. (1979) Purification,

Properties, and Kinetics of D-Ribulokinase from Aerobacter aerogenes, J. Biol. Chem., 254, in press.

432. REGULATION OF GLYCOLYSIS IN BRAIN

Basford, R. E., University of Pittsburgh, School of Medicine, Dept. of Biochemistry, *3550 Terrace St., Pittsburgh, Pennsylvania,* 15261, United States of America

The overall objective of the proposed research is to examine the possible regulation of glycolysis in brain by subcellular translocation of glycolytic enzymes. The partitioning of hexokinase, phosphoglucose isomerase, phosphofructokinase, as well as the non-glycolytic enzyme creatine kinase, between soluble and membrane-bound forms will be determined as a function of the energy status of synaptosomes and neurons. Isolated synaptosomes and neurons from rat brain will be used as a model of intact brain. Preliminary studies on neuroblastoma and glioma cell lines in tissue culture will also be initiated. The energy status of the isolated cells will be varied in a controlled manner and the subcellular distribution of the five enzymes correlated with the energy status as determined by the concentrations of ATP, ADP, AMP, creatine phosphate and glucose-6-phosphate.

433. NEURONAL REGULATION ON GLIAL BASIC MYELIN PROTEIN SYNTHESIS

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During the tenure of this project, heterokaryons were constructed between isolated active myelinating rat (21 days old) cerebral neurons and low-serum adapted rat glioma (C6) cells; between mouse neuroblastoma (2a) cells and C6 cells; and between myelinating rat neurons and non-active myelinating adult rat cerebral central ovate white matter cells. Significant increase in two of the myelination related enzyme -2', 3'-cyclic nucleotide phosphohydrolase (CNP) and UDP-galactose: ceramide galactosyl transferase (C Galt) - activities was found in the neuron-glial heterokaryons. Findings suggest that neuronal interaction probably is necessary in the myelinogenesis process.

G. OTHER METABOLIC AND BIOCHEMICAL STUDIES OF NERVOUS SYSTEM TUMORS

434. FLUOROGENIC SUBSTRATES FOR CELLULAR PROTEASES

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There is evidence to suggest that there could be an increased production of plasminogen activator associated with many transformed cells compared with their normal counterparts. Studies have continued on the synthesis of improved fluorogenic compounds that can detect different levels of the activator. In addition kinetic studies have been carried out with a number of the substrates. It appears that their kinetics of hydrolysis both with trypsin and plasmin are typical of the interaction of serine proteases with arginine esters. The hydrolysis is directly proportional to the enzyme concentration and is inhibited by p-p'-nitrophenylguanidobenzoate. The rate of hydrolysis is proportional to the concentration of urokinase in the presence of excess plasminogen. Under standard conditions normal monkey and rat kidney cell lines have no detectable activity compared to a rat brain tumor cell line. It has been shown by gel electrophoresis that the activity associated with transformed cells that is dependent upon plasminogen for the hydrolysis of the substrates is a plasminogen activator. Various ratios of transformed and normal cells have been mixed and assayed. The results show the assay to be quantitative and that the various cell lines used have a characteristic amount of plasminogen activator.

435. PLASMINOGEN ACTIVATOR ACTIVITY OF HUMAN BRAIN TUMORS IN VITRO

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OBJECTIVE: To see if the dexamethasone effect on perineoplastic oedema of brain tumors is mediated by alteration in plasminogen activator activity of the brain tumor cells.

APPROACH: Cell culture of brain tumors; assay of plasminogen activator activity (in vitro) of tumor cells with and without dexamethasone.

PROGRESS: Initial tumors tested have shown dexamethasone suppression of plasminogen activator activity.

436. REVERSE TRANSCRIPTASE IN TUMOR CELL (HUMAN MILK, TUMORS)

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OBJECTIVE: Develop and adapt methods of assay for reverse transcriptase (RT) in several human tumors of interest in Greece (i.e., breast, spleen, eye). Correlate these studies with results from morphological, pathological data.

APPROACH: Use cell cultures and fresh and/or frozen tumors or milk from genetically and geographically isolated populations in Greece. Use both biochemical and immunological assays for RT detection. Detection of RT in any of the above human material will be followed by the isolation and characteization of the enzyme.

437. REGULATION OF METABOLISM IN GLIOMA AND NEUROBLASTOMA CELL LINES

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Studies are being carried out on primary cultures of astrocytes and on several transformed glial cell lines in vitro. Primary astrocytes are derived from neonatal rats, and other cell lines were derived from rodents either by viral transformation of primary cutures (TRPG, S22) or from tumors induced in situ and subsequently established in cell culture (C6, B9, B82).

The levels of key metabolites, glucose, lactate, pyruvate, ATP, P-creatine, GABA, glutamate and cyclic nucleotides were measured in cells and medium after refeeding confluent cells with MEM plus glucose. Primary astrocytes, HSV-1 transformants derived from primary astrocytes (TRPG) and C6 cells all show a rapid increase in glucogen content after refeeding, while other glial cell lines, B9, B82 and S22, synthesize relatively low levels of glucose.

The various lines have been examined for a cyclic nucleotide response to beta-adrenergic agonists, purinergic agonists and others. All cell lines examined to date exhibit a response to adrenergic stimulation, but only the primary astrocytes exhibit a response to adenosine or adenosine analogs.

438. RETINAL TUMOR CELL LINES IN TWO ANIMAL MODELS

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Two continual retinal tumor cell lines (RAO 188 from rat and UAL 1-3 from mouse) have been established in our laboratory and are being used for in vitro and in vivo experiments. The objectives of these studies are (a) to test the hypothesis that urinary concentrations of vanilmandelic acid and homovanillic acid (the main breakdown products of catecholamine) reflect degrees of retinal tumor cell differentiation, and (b) to develop reliable criteria for the effectiveness of currently available anticancer agents. During the first year of work, we have investigated the course of maturation in retinoblastoma cells to establish background data for our planned studies. Our specific aim has been to determine what triggers dedifferentiation of the cells. We found that exposure of undifferentiated RAO 188 cells to cAMP analogs (DBcAMP and 8-BrcAMP) caused cell growth to cease and decreased DNA and RNA synthesis. This effect became irreversible after a period of 10 days exposure to the drugs. This finding will be used in our in vivo experiments. That is, before transplanting RAO 188 cells into animals (for studies of catecholamine metabolism), the cells will be incubated with cAMP analog for 10 days to ensure that they will remain differentiated in the animal system.

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mura, T., (eds.). Tokyo, Nanko-do Medical Publishers, 1979, pp. 636-653. Nakajima, T., and Mukai, N.: Cell origin of human adenovirus type 12-induced subcutaneous tumor in Syrian hamsters. Acta Neuropathol. (Berl.) 45:187-194, 1979.

439. INTRACELLULAR ENZYME KINETICS AND CARCINOGENS

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Microspectrofluorometry in conjunction with microelectrophoretic injection of metabolic intermediates is being used to follow in situ the metabolic activity of normal versus transformed or malignant cells and the intracellular distribution, interactions or metabolization of fluorescent carcinogens. Using NAD(P) yields (reversibly) NAD(P)H transients due to microinjection of glucose-6-P plus allosteric activators, the rate constants of NAD(P) reduction and reoxidation, and the relationship of overall metabolic rate to concentration of substrate introduced (So), e.g., R equals a(S)b, can be now determined in a variety of cell lines: i.e. EL2, L, NCTC 6991 normal rat fibroblasts versus NCTC 6992 transformed, NCTC 7505A normal mouse fibroblast versus NCTC 7505B transformed, CG 787 normal glia versus MG 251 glioma, HPM 73 highly melanizing melanoma versus less melanizing HPM 67. Studies on the glycolytic chain will be complemented by investigation of other pathways, e.g., pentose shunt, gluconeogenesis, mitochondrial-cytosolic interactions. The findings will be correlated to phases of cell cycle and intracellular structure. The same approach will be extended to cell lines such as the clone C3H/10 T1/2CL8 successfully used for the qualitative and quantitative studies of oncogenic transformation by chemicals, many of which are fluorescent. A grating microspectrofluorometer is being equipped with a long working phase and multioptional dichromatic high resolution mirror arrangement for simultaneous phase-fluorescence observations. Coenzyme fluorescence spectra with emphasis on elucidation of the flavin component will be related to metabolic activity or cell type. Preliminary spectral studies suggest that both the penetration and elimination of carcinogen molecules follow exponential laws. The in situ approach will be concentrated on the localization and detection of carcinogen interactions or metabolites, attempting to identify metabolites by their fluorescence spectra. Parallel topographic studies by multisite microfluorometry will aim at the detection of associated or concomitant changes of metabolic activity (or deregulations).

440. REGULATION OF INTRA- AND INTERCELLULAR COMMUNICATION

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The objective is the study of intra- and intercellular communication and metabolic cooperation, using multichannel microfluorometry in conjunction with the microinjection of fluorescent tracers (e.g., 6-carboxyfluorescein) or metabolites (e.g., glucose-6-P). Recently the emphasis has been on work with metabolites. While the microinjected metabolites themselves are not fluorescent, they lead to transient changes in the reduction-reoxidation of intracellular fluorescent coenzymes (e.g., NAD(P) yields (reversibly) NAD(P)H). In recent studies, there was no cell-to-cell transfer of metabolites in EL2 cells, normal mouse fibroblasts NCTC 7505A, mouse melanoma HPM 67 and 80% of L cells. 30% of glia CG 787 and glioma MG 251, 25% of transformed rat fibroblasts NCTC 6992 and 70% of well-differentiated mouse melanoma HPM 73 were well-communicant. Occasional weak communication was found in NCTC 6991. In these cell lines the intercellular transit time tau varied from approximately 1 sec. to 30 sec.

The current goals are the study of intercellular transfer kinetics and metabolic cooperation, in correlation to intracellular metabolic activity, intra- and extracellular ions and the topography of cells within a cluster (e.g., communication to nearest neighbors or more remote cells via intercellular processes). Towards this goal, a recently completed microscopic-optical arrangement allows simultaneous phase observations and rapid multichannel microfluorometry with an option for fluorescence photography for the definition within a cell cluster of a 'communication territory' which may be the structural basis for 'multicellular integrated states.' The role of Ca(II) in the kinetics of intercellular communication and metabolic cooperation will be evaluated using a two-injector system for separate microinjections of Ca(II) and tracers or metabolites. Clusters followed with tracers or metabolites will be retrieved for subsequent electron-microscopic studies. Thus the complex of intercommunicating cells will be defined in terms of intercellular transfer kinetics, metabolite exchange and topography of intercellular junctions.

BIBLIOGRAPHIC REFERENCES: The Intercellular Transfer of Molecules in Tissue Culture Cells: A Kinetic Study by Multichannel Microfluorometry. E. Kohen and C. Kohen. Tenth Miami Winter Symposia. Differentiation and Development, pp. 411-439. Academic Press, New York 1978.

441. DIFFERENTIATION AND STROMA-INDUCTION IN NEURAL TUMORS

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The object of this research is to study, in in vitro systems established in this laboratory and in an appropriate animal tumor model, some of the phenomena pertaining to cell differentiation, tumor maturation and stroma-induction, as expressed morphologically, biochemically and immunologically, in neoplasms of the central and peripheral nervous system. The tumors investigated include human neoplasms; experimental rat tumors obtained following transplacental ethylnitrosourea (ENU); an established glial rat tumor cell line (C-6); and the neuroepithelial component of a transplantable mouse testicular teratoma (OTT-6050) maintained in syngeneic animals in ascitic and in solid forms.

Two main approaches are used: 1. The establishment of suitable organotypic culture systems that permit the sequential study of differentiation by morphologic, immunologic and immunoradiometric methods. Autoradiographic studies are being undertaken to determine the kinetics parameters applicable to these cultures, particularly in reference to the growth fraction that can be estimated in the steady-state regions of the explants.

2. The biological properties and in vivo and in vitro behavior and manipulation of the mouse transplantable teratomas as a model for neuroepithelial neoplastic differentiation. The interesting aspect of this model lies in the model's applicability to the study of embryonal central nervous system tumors in man. A long-term objective is the production of a pure experimental tumor in which differentiation is restricted to neuroepithelial derivatives. Markers of differentiation include those concerned with gliofibrillogenesis (GFA protein), cell surface membrane antigens specific for neuroepithelial cells, melanin production in association with primitive neuroepithelium, and the determination and quantitation of biogenic amines by high-sensitivity microspectrofluorometry.

442. BIOCHEMISTRY OF SENSORY NEUROTRANSMISSION IN HAIR-CELL SYSTEMS

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The major goal of this project is to identify, using a predominantly chemical approach, the neurotransmitter or neurotransmitters that carry stimuli from the hair cells to the auditory and lateral-line nerves, and to describe the biochemical systems that make and break down these neurotransmitters. We also plan to identify materials produced by hair cells in response to high-level noise. Using high-performance liquid chromatographic (HPLC) analysis with fluorescence detection of orthophthaldialdehyde/2-mercaptoethanol adducts, we will analyze cochlear fluids in silence and in noise, and lateral-line hair cells after stimulation and non-stimulation. HPLC analysis will also be used to detect small-molecule content of synaptic vesicles from lateral-line hair cells.

In addition, polyacrylamide gel electrophoresis and other separation techniques will be used to detect enzymes and structural proteins that are specific to the sensory-cell (neuromast) fraction of the lateral line. At present we have detected about 40 primary-amine compounds in extracts of neuromasts of the

African clawed frog, and about 70 primary amine components in perilymph from the inner ear of the guinea pig. Identification of unknown compounds is now in progress.

443. IN VITRO DIFFERENTIATION OF CLONAL CELLS

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We are studying in vitro differentiation of different cell types from ethylnitrosourea-induced rat neurotumors. Biochemical cytological characterization of several cell lines clonally isolated from a rat neurotumor (RT4) is conducted on their neuronal and glial properties. We are particularly interested in the phenomenon of 'cell-type conversion' that we have discovered, in which a multipotential stem cell line converts to three different cell types in culture and these derivative cell types possess either glial (S100) or neuronal (voltage-dependent Na ion influx) properties. Population-wise, the stem cells have both properties. Several known glial and neuronal properties will be examined in these cell lines. Cytochemical analyses of the individual cells of the stem-cell population will be made. Various culture conditions that may influence the rate and direction of cell-type conversion will be examined. Another phenomenon we have noticed is 'conversion coupling,' in which a specific combination of two or more differentiated characteristics are expressed in a stem cell-derived cell type. This is extremely important for the in vitro studies of cell differentiation and will be studied in detail. Cloning of a few additional neurotumors of central and peripheral nerve origins will also be performed to establish new families of neurocell types which are related by cell-type conversion of stem cells.

444. DETERMINATION OF SPECIFIC NEURONAL ANTIGENS IN SERUM AND CEREBROSPINAL FLUID OF NEUROLOGICAL PATIENTS

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Neuron-specific enolase (NSE), a unique isoenzyme of the glycolytic enzyme enolase, is present only in neurons and is structurally distinct from enolases in other tissue. Using specific radioimmunoassay (RIA) for NSE, we have quantitated the NSE content in cerebrospinal fluid (CSF) of patients suffering from a variety of neurological disorders. Antisera to NSE do not react with enolases from other tissues and we consider it to be a specific neuronal marker for neuronal destruction in CSF in a variety of CNS disorders.

Sixty-three patients with a variety of neurological disorders, which included both benign and malignant brain tumors, metastatic tumors to the brain, dementing syndromes, Parkinson's disease, amyotrophic lateral sclerosis, strokes, herniated lumbar discs, myelopathy, and various primary neuronal degenerations, had lumbar punctures during the course of their routine evaluation. NSE was analyzed by RIA on 3 ml of CSF. The results indicate that the patients with acute, subacute, and paroxysmal disorders such as massive stroke and status epilepticus, and controls showed an increase in NSE. Patients with brain tumors did not show such an increase in NSE. This study suggests that further evaluation of the usefulness of NSE in CSF of patients with neurologic disorders is warranted. A manuscript is in preparation for the submission of these results.

445. DERIVATION OF NERVE CELL LINES FROM THE BRAIN

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We have recently succeeded in isolating neuronal and glial clones by transforming rat cerebellar cells with Rous sarcoma virus which is temperature-sensitive for transformation (ts-RSV) (Giotta, G. J., J. Heitzmann, and M. Cohn, submitted for publication). In the coming year we propose to identify the cerebellar cell types that the ts-RSV transformed cell lines represent. This will consist of 1) determining the neurotransmitter content, enzymes involved in neurotransmitter synthesis, and the types of neurotransmitter high-affinity uptake systems present in the various cell lines, and 2) the use of immunological methods to identify cell surface and cytoplasmic markers unique to specific classes of cerebellar neuronal and glial cells.

446. BIOCHEMICAL ASPECTS OF EXPERIMENTAL BRAIN TUMORS (RATS)

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This is part of a broader project. A summary of this subproject is not available.

447. CYSTATHIONINE AND VANILLYLMANDELIC EXCRETION IN NEUROBLASTOMA (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

VII. CYTOLOGICAL AND OTHER BIOLOGICAL AND BIOCHEMICAL STUDIES OF NERVOUS SYSTEM TUMORS

A. CYTOGENETIC STUDIES

448. CYTOGENETICAL ANALYSES OF HUMAN TUMORS

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AIM: Studies by banding methods of the chromosomes in colonic tumors, lymphomas, exudates, cell lines of malignant gliomas and breast carcinomas.

PROJECT 1: Twenty-three non-Hodgkin's and non-Burkitt's lymphomas have been analyzed so far. One third of these and those reported in the literature showed a 14q positive marker. Three further anomalies of recurrent type have been observed and these results are to be published.

PROJECT 2: Successful results have been obtained from 4 colonic adenocarcinomas. The results will be reported when more cases have been collected.

PROJECT 3: Seven cell lines from malignant gliomas have been analyzed. The karyotypic profile was distinguished by an increased proportion of Nos. 7 and 19 and reduction of Nos. 4 and 22, and most likely also Nos. 10 and 12. Three types of recurrent marker chromosomes were seen. Next to be studied are clones derived from cell lines of malignant gliomas.

PROJECT 4: Two hundred and fifty exudates have been studied by chromosome methods. Cytogenetical analyses were shown to be an extremely reliable method of determining whether the effusion is caused by malignancy. Monosomy 14, monosomy 22 and 13q negative were three anomalies found in two malignant mesotheliomas. The studies are to be continued.

PROJECT 5: Four mammary carcinomas have been studied. None of them contained any marker wholly or partly derived from the long arm of No. 1 - an anomaly which has been proposed to be specific for breast carcinomas (according to results from exudates ad cell lines from exudates). Further studies are needed to clarify this important question.

449. CHROMOSOME STUDIES IN RETINOBLASTOMA

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We are presently studying 89 patients with retinoblastoma and 211 of their family members cytogenetically. We are looking for an interstitial deletion of chromosome 13 and for evidence of chromosome fragility. We have concluded a study using markers on chromosome 13 to follow the segregation of that chromosome with the occurrence of familial retinoblastoma. Meiotic crossing-over occurred with too great a frequency for these polymorphic markers to be used to predict those at risk for developing retinoblastoma.

450. RETINOBLASTOMA AS A MODEL FOR HUMAN ONCOGENES

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A mutation on chromosome 13 has been implicated in the heritable form of bilateral retinoblastoma. We have observed numerous chromosome alterations in fresh tumors removed from patients with retinoblastoma; these alterations, involving translocations and deletions, affect different chromosomes in different tumors and seldom involve chromosome 13. Although the mutation predisposing to retinoblastoma may be on chromosome 13, actual tumor development may require variable changes in genes on other chromosomes. To compare the genetic defects in tumors from different patients, we are using a somatic cell genetic approach to map the genes required for malignant transformation. Retinoblastoma tumor cells have been hybridized with 'normal' rodent fibroblasts and hybrids selected which express a malignant phenotype, as measured by their ability to form tumors in nude mice. Since such hybrids randomly lose human chromosomes with no selective pressure, it should be possible to correlate specific chromosome changes with the malignant phenotype.

451. HUMAN GENE MAPPING IN NEUROBLASTOMA CELL HYBRIDS

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Genes coding for the specialized products of differentiated mammalian cells have in general been difficult to map. To date none of the genes specifying enzymes involved in neurotransmitter metabolism have been assigned to specific chromosomes. We have a cell system that is anticipated to provide the means for mapping the human structural gene for tyrosine hydroxylase, the presumed rate-limiting enzyme for the biosynthesis of the catecholamine neurotransmitters dopamine and norepinephrine. Human neuroblastoma cells expressing high levels of tyrosine hydroxylase activity have been fused with mouse neuroblastoma cells that do not express this activity. Hybrid clones, selected with use of a positive selection system for the gene of interest (based on ability of cells with tyrosine hydroxylase activity to grow in medium devoid of the essential amino acid tyrosine), have measurable levels of the hydroxylating enzyme. Initial Giemsa-banding karyotype analyses showed preferential segregation of human, not mouse, chromosomes. Currently available human-mouse neuroblastoma cell hybrids and planned hybridizations involving human neuroblastoma clones are expected to enable us to: (1) Assign a human chromosomal location for the tyrosine hydroxylase gene; (2) determine whether the long, homogeneously staining region, HSR, a unique metaphase chromosome abnormality characterizing many continuously cultured human neuroblastoma lines, may be associated with expression of the neuronal phenotype; (3) determine whether expression of L-amino acid decarboxylase or dopamine-beta-hydroxylase activity is correlated with expression of tyrosine hydroxylase. In addition to new knowledge of gene mapping and gene expression, the proposed study may provide new insights into regulation of catecholamine biosynthesis in these human tumor cells.

452. CLONAL KARYOTYPIC EVOLUTION IN HUMAN SOLID TUMORS

Trent, J. M., Salmon, S. E., Davis, J. R., University of Arizona, School of Medicine, Dept. of Internal Medicine, *1501 N. Campbell Ave., Tucson, Arizona*, 85721, United States of America

Detailed cytogenetic banding analysis will be applied to a variety of human cancers to directly assess clonal karyotypic abnormalities. Sequential chromosome studies will be conducted using a new cytogenetic procedure which samples tumor cells grown in an in vitro bioassay capable of supporting colony formation from a wide variety of human cancers. Specific time points for sequential analysis will be: 1) at diagnosis (prior to therapy); 2) following cessation of therapy (if measurable residual disease); 3) at relapse. Patients with solid tumors and malignant effusions from breast, ovary, and lung cancer or patients with neuroblastoma will be studied. The objectives are to assess the clonal karyotypic evolution in primary and metastatic lesions as a response to time and treatment.

Additionally, the karyology of tumor colony-forming cells exposed but resistant to in vitro incubation with various chemotherapeutic agents will be compared to unexposed control cultures. Selective action of these drugs on karyotypic clonal expansion or contraction will be assessed and results compared to measurable in vivo selection at the time of recurrance of disease. Finally, specific probes for subsegments of chromosomal DNA will be employed to further cytogenetically characterize drug resistant clonogenic populations.

453. CYTOGENETIC STUDIES

Whang Peng, J., Douglass, E., Knutsen, T., Lee, E., Bunn, P., Levis, W., Broder, S., Magrath, I., Glatstein, E., Blaese, M., Li, F., Seiber, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cytogenetic Oncology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The Areas of Investigation: 1. Cytogenetic studies of human neoplastic, hematological, and congenital disease. 2. In vitro cytogenetic studies of direct tumor material, tissue culture lines, and colony cultures derived or established from patients with Burkitt's lymphoma, small cell carcinoma of lung, and cancer of the brain, ovary or testis. 3. Early detection of secondary leukemia in patients with non-Hodgkin's lymphoma, CLL, small cell carcinoma of lung using chromosomal abnormalities as markers. 4. Detection of sister chromatid exchanges in families with ataxia telangiectasia, Bloom's syndrome, and a high risk of cancer.

BIBLIOGRAPHIC REFERENCES: Douglass, E.C., Magrath, I.T., Lee, E.C., and Whang-Peng, J.: Cytogenetic studies in non-African Burkitt's lymphoma. Blood 5: 148-155, 1980. Whang-Peng, J. and Knutsen, T.: Lymphocytic leukemias, acute and chronic. In Penington, D.C. (Ed.): Clinics in Haematology, 1980, Vol. 9, pp. 87-127.

454. STUDY OF MALIGNANCY IN HUMAN TUMOR CELL LINES

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Cell lines from two presumed genetic human tumors - neuroblastoma and retinoblastoma - will be used to study several aspects of oncogenesis. Chromosome analysis of the tumors and cell lines will be performed to determine if non-random chromosomal changes are characteristic of each tumor type. The functional significance of two chromosome abnormalities already described in these tumors - the Homogeneously Staining Region and double minute chromosomes - will also be analyzed. Somatic cell hybridization (involving the fusion of the tumor cells with other malignant and non-malignant cells of the same or different species) and the technique of gene mapping will be used to study the pattern of inheritance of the malignant phenotype in these tumors and to assign the genes involved in the regulation of malignancy to individual chromosomes.

455. IN VITRO AND IN VIVO STUDIES OF RETINOBLASTOMA

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The establishment of a number of cultured cell lines of retinoblastoma during the past several years has opened the way for a new program of research with this malignancy. It has made possible a short-term, but effective and dependable, ocular model of the disease in immunosuppressed rabbits. Various retinoblastoma cell lines will be studied in vitro and in our animal model for the range of cellular sensitivity to the photodynamic activity of natural and synthetic porphyrins such as hematoporphyrin and tetraphenylporphine sulfonate. Retinoblastoma karyology of the tumor, and of family member lymphocytes, will be examined for possible distinguishing chromosome abnormalities. Cell lines will be used in animal studies to determine their ability to develop metastatic spread. They will also be examined for antigenic and biochemical characteristics that may help establish the cell of origin in the retina.

BIBLIOGRAPHIC REFERENCES: Sery, T.W.: Photodynamic Killing of Retinoblastoma Cells with Hematoporphyrin and Light. Cancer Research 39:96-100, 1979.

456. CYTOCHEMICAL ANALYSES OF THE MAMMALIAN GENOME

Arrighi, F. E., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept.

of Biology, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

OBJECTIVE: The objective of this project is to ascertain whether specific chromosomal changes are associated with a specific class of neoplasms. Detailed analyses of the chromosome abnormalities will be made on each neoplastic cell population.

APPROACH: Although we shall analyze the chromosome constitution of whichever human tumors become available, we shall concentrate mainly on melanomas, breast cancer and neuroblastomas.

Specimens obtained directly from the patients and also the established culture lines will be used for the cell morphology, growth characteristics and chromosome number and variability. Q-, C- and G-banding will be performed on each sample in order to establish the chromosome characteristics. Electron microscopy will also be employed for certain chromosome types.

457. BIOCHEMICAL AND CYTOGENETIC MARKERS IN RETINOBLASTOMA

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The linkage between esterase and the retinoblastoma gene on chromosome 13 is being investigated. Various human retinoblastomas from both sporadic and hereditary cases are being propagated intraocularly and subcutaneously in the nude mouse. Esterase D activity and the electrophoretic pattern are being investigated in the primary tumors and those grown in the nude mice. Similarly the chromosome pattern of each tumor is being evaluated using the trypsin-Giemsa banding technique. In addition, blood samples from patients with both hereditary and sporadic retinoblastoma are being evaluated for their esterase D activity. This is a large scale study involving patients throughout the country. Dr. Robert Sparkes at UCLA Medical School is collaborating on this project.

458. GENETIC STUDIES OF RETINOBLASTOMA AND THE RB-1 LOCUS

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The objectives of this proposal are to determine the precise relationship between the genetic locus coding for the enzyme Esterase D and the RB-1 locus, defined by an interstitial deletion of chromosome 13, on human chromosome 13 by quantitative assays of Esterase D and karyotype analysis of members of families with multiple generations of retinoblastoma patients. To determine whether the quantitation of Esterase D is a useful tool in searching for deletions of chromosome 13 not visible during karyotype analysis, and to apply this to individuals with retinoblastoma in the absence of an obvious deletion. To search for evidence for the involvement of more than one genetic locus in the development of retinoblastoma by linkage analysis between RB-1 and a large battery of genetic markers as a test of the multi-hit hypothesis for childhood cancer.

459. PATHOGENETIC DETERMINANTS OF HUMAN CNS TUMORS

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Our major aim is to understand the changes in nuclei of aneuploid CNS neoplasms. Isolation and characterization of subsets of repeated sequences in mouse and human DNA will be continued, with emphasis on chromosomal structure (studies by in situ hybridization) and DNA analysis. Ultrastructural studies of the nucleolus organizing regions in interphase nuclei will be correlated with quantitative analysis of DNA restriction profiles from normal and neuroectodermal tumor cells, using defined ribosomal DNA probes. Methods to analyze other DNA sequence subsets of interest, with respect to their three-dimensional arrangement in chromosomes are currently being investigated.

460. DETAILED CELL KINETIC ANALYSES OF HUMAN NEUROBLASTOMA

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The overall goal of this project is to increase our basic knowledge of the biology of cell replication in human neuroblastoma. Two models will be studied: the first consists of several human neuroblastoma lines in vitro and the second consists of these neuroblastoma lines grown in solid tumor form in the immunodeficient nude mouse. These tumors will be examined for synchrony, diurnal variations, and changes in proliferative characteristics with increasing size. The classic cell kinetics measurements, such as labelling and mitotic indices, and fraction-labelled mitoses curves, will be performed to determine baseline cell cycle times and parameters. Several newer techniques will be used also. First, flow cytofluorometry will be used to study overall changes in DNA distributions within the tumors. Combined tritiated thymidine autoradiography and DNA cytofluorometry will be used to provide independent measurements of DNA synthesis status and cell cycle position to study the kinetic characteristics of proliferating and non-proliferating cells. These studies will provide a detailed analysis of the cell kinetics of untreated neuroblastoma in order to provide a baseline with which treatment can be rationally formulated and against which therapeutic efforts can be objectively compared.

BIBLIOGRAPHIC REFERENCES: Neely, J.E.: Constancy of Cell Kinetic Parameters in a Human Neuroblastoma Line in the Nude Mouse, in Recent Advances in Neuroblastoma Research (A.E. Evans, ed.), 1980, in press.

461. CYTOGENETIC AND TRANSPLANTATION STUDIES OF NEUROBLASTOMA (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

462. CYTOGENETIC AND TRANSPLANTATION STUDIES ON NEUROBLASTOMA CELL LINES (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

463. CHANGES IN POTENTIAL BRAIN TUMORS - CYTOGENIC AND ONCOGENIC TRANSFORMATION (RAT)

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This is part of a broader project. A summary of this subproject is not available.

464. DAMAGE AND REPAIR OF CHROMOSOMAL DNA IN BRAIN TUMOR (RATS)

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This is part of a broader project. A summary of this subproject is not available.

B. CYTOCHEMICAL STUDIES

465. THE CYTOCHEMISTRY OF THE NERVOUS SYSTEM

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This program has two long-range objectives. One is to study the nervous system and brain tumors with quantitative histochemical methods. The other is to improve and expand the system of quantitative histochemistry which was developed to meet the first objective, but which has proven useful in the study of other complex organs (kidney, adrenal gland, liver, pituitary gland, pancreas, skeletal muscle, plant tissues).

Samples weighing as little as one nanogram are dissected from frozen dried sections, weighed and analyzed with the aid of enzymatic sequences terminating in an NAD or NADP step

The final pyridine nucleotide product is amplified by enzymatic cycling to give virtually unlimited sensitivity. Early steps are carried out in submicroliter volumes under oil. For the projects comtemplated, a number of new methods will need to be added to the already long list of those available for enzyme, metabolite and cofactor measurement.

Projects proposed include the determination of the levels in human brain tumors and the distribution in retina and brain of (1) cyclic nucleotide phosphodiesterase isozymes, and adenylate and guanylate cyclases, (2) enzymes of adenylate and guanylate metabolism, and (3) glycoproteins.

466. CYTOPLASMIC DETERMINATION OF DETAILED CELL MORPHOLOGY

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The characteristic morphology of cells is controlled in information in the genome, and expressed through the organization of cytoplasmic structures and the cell surface. The underlying molecular mechanisms of that expression are not understood. Some transformed cells which do not display differentiated shape can be induced to spread and take their typical morphology. Several results strongly suggest that the components and information necessary for assumption of differentiated morphology can reside in the cytoplasm. We have recently used such a cell system to study specification of morphology at the cytoplasmic level. Mouse neuroblastoma, an established line of transformed cells, grow round in culture, but they can be induced to extend axon-like neurites and so resemble their normal counterparts. The patterns of neurites form an extremely wide repertoire of morphologies. However, the majority of mitotic sister cells are uniquely related to one another in considerable detail. The results suggest that sister cells share determinants of specific cell shape. This system presents a striking and easily visualized example of morphological control at the cytoplasmic level. We will use it as a tool to study the structural and molecular basis for the storage, expression and mitotic transmission of the morphological determinants. In particular, we will determine the persistence of the determinants through subsequent rounds of cell division. We will address the role of the cytoskeleton - microtubules and microtubule organizing centers, microfilaments, and intermediate filaments - using specific drugs and microscopic techniques. We will examine the influence of external material - components of the cell membrane or attached to the substrate - in guiding neurite outgrowth. These experiments will address a fundamental question in cell and developmental biology, with relevance to the transformed phenotype: how are cellular components organized to produce a particular morphology?

467. MICROTUBULE INITIATION SITES AND THE CYTOSKELETON

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The long-range objective of this proposal is twofold; first, an understanding of how the spatial distribution of microtubules in the cell is achieved, and the biochemical nature of microtubule organizing centers and second, an ultrastructural description of the arrangement and interactions of the three major fibrous systems of the cell. We plan to extend to an ultrastructural level our earlier studies of multiple microtubule organizing centers in fibroblast and neuroblastoma cells to answer the following question; what happens to the sites in the transition from interphase to mitosis, what is the polarity of microtubule growth and what is the structure of these sites to determine their composition and specifically to ask whether the sites contain tubulin, and if they do, to ascertain the form of tubulin. Finally, we hope to be able to distinguish structurally the classes of sites found in fibroblast and epithelial cells. Using a newly developed ultrastructural method for viewing cytoskeletons (and developed in collaboration with John Heuser, UCSF) involving rapid freezing, freeze-drying, and production of rotary shadowed platinum replicas we plan to investigate the surface and lumen of microtubules for binding sites of associated proteins and study the mechanism of microtubule elongation. Using this technique, we plan to describe the actin, intermediate filament, and microtubule distribution in fibroblast and differentiating neuroblastoma cells.

468. BIOCHEMISTRY OF NEUROFILAMENT PROTEIN AND REGULATION OF BRAIN FILAMENTS IN VITRO

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The main purpose of this project is to investigate the role of neurofilament proteins in normal and pathological conditions. The isolation of GFA protein from bovine brain filaments by immunoaffinity chromatography (Biochim. Biophys. Acta 578, 305, 1979) has provided the definitive proof that a major polypeptide at approximately 50,000 daltons in this preparation is gliofilament and not neurofilament protein as repeatedly reported in the literature. A simple immunohistological assay has been established for the assessment of anti-neurofilament activity in immune sera, i.e. the selective staining of Purkinje cell baskets and parallel fibers in the lower half of the molecular layer of the cerebellum. These are markedly enriched in neurofilaments as compared with the adjoining structures. Antisera have been raised against two neurofilament polypeptides at approximately 70,000 and 150,000 mol. wt. The data presently available in the laboratory indicate that these polypeptides are biochemically and immunologically non-related (FEBS Lett. 103, 144, 1979; Soc. Neurosci. Abstr. 5, 399, 1979). The preparation of antisera to different neurofilament components is now providing the first experimental data suggesting that neurofilaments accumulating in pathological conditions are biochemically abnormal. The in vitro expression of neurofilament protein has been demonstrated in neuroblastoma cells (Develop. Neurosci. 1, 142, 1978). Preliminary observations in primary monolayer cultures of dissociated brain cells indicate that neurofilament antisera may provide a powerful tool for the identification of neurons in culture.

469. CONTROL OF GLIAL FIBRILLARY ACIDIC PROTEIN FORMATION IN HUMAN BRAIN TUMOR (GLIOMA) CELLS

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This proposal is designed to study glial fibrillary acidic protein (GFAP) in human glioma cells, to demonstrate factors that effect the formation of the protein, to relate GFAP formation to growth characteristics of glioma cells in culture, and to examine whether any of these characteristics relate to the clinical course or therapeutic response of the donor. GFAP is a specific marker for astrocytes and is believed to be associated with the 9 nm cytoplasmic filaments. The protein will be localized by the immunoperoxidase method and quantitative determinations done by rocket immunoelectrophoresis. Six factors, known to affect glioma cell growth or differentiation, will be studied for their putative effects on GFAP formation. The factors to be studied include polyamine metabolism, low serum concentration in culture media, dimethylsulfoxide, butyric acid, cyclic 3'5 AMP, and amethopterin.

470. CORD REGENERATION WITH CLINICAL CORRELATION AND HETEROTOPIC OSSIFICATION IN SPINAL CORD INJURY (ABBREV)

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The project is directed at investigating the glial response in CNS injury and particularly its role in nerve regeneration. The development of a procedure for the isolation of non-degraded GFA protein from aqueous extracts of bovine brain has allowed experimentation resulting in the in vitro formation of filaments (J. Mol. Biol. in press). This demonstrates conclusively that GFA is the subunit of glial filaments and also provides the first piece of evidence suggesting that 100 A filaments may assemble-disassemble in vivo. The reactivity of Muller glia has been investigated in the rat retina by immunocytochemical methods. It was shown that retinal glia are comparable to CNS astrocytes, i.e. they accumulate GFA as a response to injury (Exp

Eye Res. 28, 63, 1979). In vitro studies have demonstrated that the aggregating culture system (see 1978 Summary) enhances GFA expression in C6 glioma (Experientia, in press). Also, that isolated cells are GFA positive in monolayers of G26 glioma, a cell line producing myelin glycosphingolipids (Brain Res. 163, 353, 1979). Peripheral nerve implants in spinal cord allowed a more direct approach to CNS regeneration. Neurofilament antisera developed for another project in the SCI Research Laboratory provided an essential tool, due to the intense immunoreactivity of regenerating fibers (Soc. Neurosci. Abstr., 5, 727, 1979). Collaborative studies have shown the following; 1. the distribution of GFA and myosin in brain is essentially the same (Cell Tiss. Res. 191, 493, 1978); 2. human glioma cells in vitro contain both glial-specific (GFA) and non-specific filaments (Acta Neuropath. 47, 71, 1979); 3. the practical use of GFA antisera for brain tumor diagnosis (Cancer, in press).

C. NEURORECEPTOR STUDIES

471. CELL SURFACE MEMBRANES AND RECEPTORS OF NORMAL AND TUMOR CELLS

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OBJECTIVE: To localize and characterize glycoproteins and hormone receptors on the surface of normal and tumor cells.

APPROACH: Various plant lectins (concanavalin A, wheat germ agglutinin and Ricinus communis agglutinins) and polypeptide hormones are tagged with either radioisotopes, fluorescent dyes, or polymeric microspheres. These reagents are used to detect, quantitate and map the distribution of glycoproteins and hormone receptors on the surface of neuroblastoma cells and normal and tumor adrenal cells by radio-scintillation methods, as well as light and electron microscopic techniques. The effect of labeling on the distribution and morphology of the cells is being investigated. This will be correlated with the biochemical properties of glycoproteins isolated from cell surface membranes of these cells.

PROGRESS: Glycoproteins which bind concanavalin A and wheat germ agglutinin are densely distributed on the surface of differentiated and undifferentiated neuroblastoma cells. Redistribution and endocytosis of lectin binding glycoproteins occurs when cells are labeled at 37 degrees C. The mode of redistribution is different for Con A-binding glycoproteins than for WGA-binding proteins on the cell surface.

472. ALPHA-TOXIN - CHEMISTRY AND ACTION ON MEMBRANES

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Studies will be continued in an effort to elucidate the structure and mechanism of action of staphylococcal alpha-toxin, an extracellular, hemolytic protein produced by most strains of staphylococcus aureus. Specifically, it is proposed to continue studies to characterize the membrane bound, high affinity receptor protein for alpha-toxin found on rabbit red blood cells. A second aspect will be to investigate cultured neuroblastoma cells, whole nerves and diaphragm muscle for their capacity to specifically bind alpha-toxin and to be injured by the toxin. Further, it is planned to continue studies on the CNBr peptides and eventually to establish the complete amino acid sequence of alpha-toxin that are involved in membrane binding, membrane damage and in antibody binding.

473. BIOCHEMICAL APPROACHES TO OPIATE ADAPTATION

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The proposed research is designed to identify the molecular mechanism by which mouse neuroblastoma x glioma hybrid cells adapt to prolonged exposure to opiates and other chemical stimuli. Agonist binding to opiate receptors elicits an initial, rapidly reversible inhibition of adenylate cyclase (AC) activity. Further exposure to the agonist leads to a compensatory, slowly reversible increase in activity. Removal of the agonist at this point eliminates the initial inhibition, but not the compensatory activiation, resulting in elevated c-AMP levels. These molecular events are thought to be a key factor in cell transformation.

There is good evidence that a reversible covalent modification is involved in the long-term modulation of AC activity based on a similar adaptive mechanism in bacteria and suggestive evidence in eucaryotic systems. A modification of this nature, correlated to adaptation and AC activity will be investigated. Similar but opposite modulation of cyclase activity by prostaglandin E1 and beta-adrenergic receptors as well as nucleotide and ion modulation of AC-receptor interactions will be studied.

474. CHOLINERGIC AND ANTICHOLINERGIC AGENTS

Van Scott, E. J., Temple University, School of Medicine, Dept. of Dermatology, *3322 Broad St., Philadelphia, Pennsylvania,* 19122, United States of America

This is part of a broader project. A summary of this subproject is not available.

475. TRANSCUTANEOUS NEURAL STIMULATION IN CANCER PAIN

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This is part of a broader project. A summary of this subproject is not available.

476. NEURONAL CONNECTIONS, SYNAPTIC FUNCTION AND PLASTICITY (ANNELIDS)

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This is part of a broader project. A summary of this subproject is not available.

D. HISTOPATHOLOGICAL STUDIES

477. MORPHOLOGY AND IMMUNOCYTOCHEMISTRY OF A TRANSMISSIBLE SPONTANEOUS MURINE ASTROCYTOMA

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OBJECTIVE: Recently a spontaneous, predominantly astrocytic tumor - transmissible into syngeneic animals - has been described in the VM strain of mice (Frazer, 1971; 1974). Cells from these tumors can also be maintained in vitro and produce tumors when injected into the brains of syngeneic mice. These gliomas fulfill the criteria of a good astrocytoma model: short latency, infiltrative growth, reproducibility and morphological similarity to human astrocytomas. However, little work has been done to characterize the morphology, enzyme pattern and growth properties of these tumors during development. Thus the aim of this project is to establish a reproducible, astrocytic brain tumor in VM mice and to study its cell biology. This model may be used for chemo-, radio- and immunotherapeutic studies. Frazer, H. (1971) Astrocytomas in an inbred mouse strain J. Path. 103 286-270; Frazer, H. (1974) Spontaneous astrocytomas in inbred mice. Proc. VIIth Int. Cong. Neuropath.

APPROACH: Cells originally obtained from a spontaneous murine brain tumor are being maintained in vitro and are injected intracerebrally into syngeneic mice in order to obtain a constant and reproducible brain tumor model. The growth and development of the tumors are being assessed, following sequential sampling, by light and electron microscopy. The localization of enzymes and the distribution of glial specific protein markers are also being studied.

478. HISTOLOGICAL, FINE STRUCTURAL AND CYTOCHEMICAL STUDIES OF CHEMICALLY INDUCED BRAIN TUMORS IN THE RAT

Lantos, P. L., Pilkington, G. J., University of London, Inst. of Psychiatry, Dept. of Neuropathology, *Senate House, London WC1E 6BT, England, United Kingdom*

OBJECTIVE: To assess the morphology and enzyme cytochemistry of chemically induced cerebral tumors in the rat and to study the development and possible origins of these tumors.

APPROACH: Brain tumors are produced by single intraperitoneal injection of N-ethyl-N-nitrosourea into pregnant BD 1X rats. The offspring develop gliomas which are studied by light and electron microscopy, together with a variety of cytochemical techniques for the localization of enzymes and immunocytochemical techniques to demonstrate glial specific proteins.

479. THE BIOLOGICAL BEHAVIOR AND ULTRASTRUCTURAL CHARACTERISTICS OF BRAIN TUMORS

Roytta, R. M., Frey, H. J., Torma, T., Sourander, P., Turun Yliopisto, Dept. of Pathology, *Kiinamyllynk 8-10, SF20500 Turku 52, Finland*

OBJECTIVE AND APPROACH: The aim of the first part of the investigation is to study the ultrastructural changes in different brain tumors and, in selected cases, to use nerve tissue cultures and scanning electron microscopy to further clarify the biological behavior and surface morphology of brain tumors.

The second part consists of a morphological (light microscopy) study of atypical meningomas based on the material from the Sahlgren Hospital (Department of Neuropathology) which has been collected during the last 20-30 years. The aim of this study is to look for the possible morphological changes which influence the prognosis (i.e. recurrence) of these tumors.

PROGRESS: Brain tumor material has been collected in Turku and samples have been taken for nerve tissue culture and electron microscopy. Furthermore, the growth characteristics and electron-microscopical findings obtained with nerve tissue culture are studied. In some cases surface morphology and brain specific proteins (e.g. glial fibrillar acidic protein) are also studied.

For the study of atypical meningomas all the atypical cases are collected and by using routine light microscopical procedures the common findings in relevance to recurrence (e.g. necrosis) are studied. From the beginning of 1980 samples are studied by electron microscopy.

480. MORPHOMETRIC AND DENSITOMETRIC ANALYSIS APPLIED TO CELL TRANSFORMATION AND FOR DIFFERENTIATION 'IN VITRO'

Parodi, S., Chiabreara, A., Nicolini, C., Brambilla, G., Viviani, R., Carlo, P., Beltrame, F., Universita degli Studi di Genova, Istituto de Oncologia, *Viale Benedetto 15, Pad. B, 16132 Genoa, Italy*

OBJECTIVE AND APPROACH: To try to distinguish 'normal' and 'morphologically transformed' or 'morphologically mutated' colonies with techniques of scanning microscopy plus objective densitometric and geometric analysis. To try to replace subjective judgments on colony morphology with an objective classification, based on a set of measured parameters.

PROGRESS: From preliminary work performed in a laboratory associated with our group, (Dr. C. Nicolini -Div. of Biophysics, Temple University, Philadelphia), the analysis of CHO colonies with different morphologies seems to suggest that the approach is clearly more sensitive than the human eye-brain system, plus more objective and quantitative. A.T.A.S. Leitz system is now becoming operating (in line with a minicomputer) for this approach also in Genoa.

BIBLIOGRAPHIC REFERENCES: S. Parodi, G. Brambilla, S. Lessin, F. Beltrame, C. Nicolini, Cellular morphometry in aging, differentiation, and transformation, in Chromatin Structure and Function, C. Nicolini Ed., Plenum Press (N.Y. 1978). C. Nicolini, F. Beltrame, S. Lessin, S. Parodi, Analytical cytology as possible screening method for chemical carcinogens. Proceedings of The International Workshop 'Short-Term Tests for Prescreening of Potential Carcinogens', Feb. 6-8, 1979, Santa Margherita Ligure (Genoa).

481. LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE EIGHTH CRANIAL NERVE IN RELATIONSHIP TO THE SOLITARY SCHWANNOMA

Neely, J. G., Armstrong, D., Igarashi, M., Baylor College of Medicine, School of Medicine, Dept. of Otorhinolaryngology & Communicative Sciences, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

The specific aim of this project is to establish basic neuroanatomical correlates of solitary Schwannomas of the eighth cranial nerve in man by (a) quantitating the myelinated eighth nerve fibers present in cases of solitary Schwannomas of the eighth nerve and (b) analyzing the fiber count data against preoperative auditory and vestibular test results for the identification of any significant correlation.

The project is performed by taking biopsies at the following three specific sites during resection of the solitary Schwannoma of the eighth nerve: (1) the superior vestibular nerve, (2) the cochlear nerve, (3) the trunk of the eighth cranial nerve immediately lateral to the pons. The biopsies are processed for electron microscopy and one micron thick sections are prepared for light microscopic study and the development of 400x photographic montages for fiber count analysis and for the identification of areas for electron microscopic definition.

The numbers of myelinated nerve fibers are counted from the photographic montage in each of the three biopsy specimens. Electron microscopic identification of any additional myelinated axons will be added to the count.

In order to determine the extent to which numbers of axons present can be predicted from preoperative auditory and vestibular tests, scattergrams are constructed in which the functional relationship between the fiber count and each preoperative auditory and vestibular test is plotted. Linear relationships and non-linear relationships are further analyzed with appropriate parametric or non-parametric methods.

482. MALIGNANT POTENTIAL OF UVEAL MELANOMAS

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Our objective is to develop an objective and reproducible method for determining the malignant potential of uveal melanomas from histopathologic examination. In order to achieve this objective, we will measure the cytologic features from the microslides of several hundred uveal melanomas selected from the registry of Ophthalmic Pathology (Armed Forces Institute of Pathology). After comparing these measurments with the clinical course of the patients from whom the tumors were removed, we will select those cytologic features that are useful for predicting death from metastasis. With this information we will develop objective criteria for estimating the malignant potential of newly diagnosed cases of uveal melanoma. Ultimately, concepts derived from the study of this relatively controlled neoplastic system will be applied to other neoplasms in an attempt to predict their malignant potential.

483. MYXOPAPILLARY EPENDYMOMA

Henry, J. M., Earle, K. M., Genarelli, T., U.S. Dept. of Defense, Armed Forces Inst. of Pathology, *c/o Walter Reed Medical Ctr., Washington, District of Columbia,* 20306, United States of America

Myxopapillary ependymomas occur predominantly in the region of the filum and conus medullaris. They are characterized by a typical histologic pattern and are often amenable to resection due to their location in the surgically expendable region of the filum terminale. Their presence is often obscured due to the similarity of clinical signs and symptoms to the differential diagnosis of herniated nucleus pulposus.

We have reviewed the clinical records and histologic patterns of more than 100 cases of myxopapillar ependymoma; follow-up information is available for more than 50% of these cases. Ultrastructural definition of the neoplasm is still in progress.

Based on this review, we have described certain clinical features which direct the clinician's attention to the possibility of myxopapillary ependymoma in patients presenting with the syndrome of herniated disc. We also have adduced evidence that this glial neoplasm is associated with a favorable prognosis. Various histologic variants have been defined.

484. NERVE FASCICLE LIGATION AND NEUROMA FORMATION

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Fascicle ligation for neuroma pain in 8 patients has produced relief of pain in all cases since operation: 43 mths (in 2 patients), 41 mths, 33 mths, 20 mths, 14 mths, 10 mths and 9 mths. Light and EM studies of human painful and non-painful

amputation neuromas continue to support the observation of more unmyelinated axons in painful than in non-painful neuromas. Neuroma formation in rats, cats and monkeys has shown grossly a markedly decreased bulk in the neuromal area of nerves sectioned, doubly ligated, and careful cauterization as compared to neuromas formed after cutting with or without whole nerve ligation. Light and EM studies indicate a paucity of axons in the area of the fascicle ligation and cauterization as compared to the number in the neuroma formed after nerve cutting with or without whole nerve ligation. Evidence indicates that by preventing rupture of the perineurium axons are thereby contained which limits neuroma formation and maintains an orderly arrangement of nerve fiber bundles.

485. GANGLION CELL TUMORS OF THE CENTRAL NERVOUS SYSTEM

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Ganglion cell tumors of the central nervous system (CNS) present as relatively well-localized, space-occupying lesions which are amenable to surgical excision. They are characterized by a histologic pattern of low-grade (Pilocytic) astrocytoma matrix containing a variety of immature and mature neurons and neuronal precursors. Gangliogliomas are rare lesions and little is known in reference to their biological behavior and prognosis.

We have reviewed the clinical, gross, and microscopic features of more than 60 gangliogliomas and have achieved a 50% follow-up. In addition to special stains and electron microscopy, we plan to investigate selected examples with glial-fluorescent-antibody techniques.

Gangliogliomas are tumors of juveniles and young adults; based on our studies, they generally behave as hamartomas rather than astrocytomas. Long-term prognosisis excellent.

486. NEURAL TUMORS OF THE SKIN INCLUDING PLEXIFORM NEUROFIBROMAS, MYXOID NEUROMAS AND NEUROMAS

Helwig, E. B., Gallager, R. L., U.S. Dept. of Defense, Armed Forces Inst. of Pathology, *c/o Walter Reed Medical Ctr., Washington, District of Columbia,* 20306, United States of America

The purpose of this study is to identify the pathologic changes and clinical behavior of neuromas of the skin.

We described 53 examples of unreported benign tumor of nerve sheath origin occurring in the dermis. Tumors were usually initially noted by patients in the first or second decade, and topographically there was a predilection for the central area of the face, the arms, and the shoulders. There was a 4.3/1 female to male predominance among patients. Histologically, these lesions contained nests and cords of large cells in a variably mucinous matrix. In a number of instances, a close histologic relationship existed between tumor cell and small nerves. Despite nuclear atypia and a variable number of mitotic figures, the tumors have behaved in a benign fashion. Only two to our knowledge recurred, and inadequate excision was the apparent cause. We propose the name 'neurothekeoma' for these tumors.

A manuscript has been submitted to the American Journal of Clinical Pathology.

487. SARCOMAS OF THE BRAIN AND SKULL

Henry, J. M., McGee, T. P., Armbrustmacher, V. W., U.S. Dept. of Defense, Armed Forces Inst. of Pathology, *c/o Walter Reed Medical Ctr., Washington, District of Columbia,* 20306, United States of America

It is notoriously difficult to evaluate the biological activity and prognosis of meningiomas based on histologic pattern. Most meningiomas recur with time, and recurrence is determined largely by surgical accessibility and ease of removal, rather than by histologic pattern.

We have retrieved more than 100 cases of atypical meningiomas and meningeal sarcomas characterized by aggressive growth behavior, rapid recurrence, local invasion and metastatic activity. In reviewing these cases, we are attempting to correlate histologic features, including degree of cellularity, necrosis and mitotic activity with evidence of rapid and aggressive growth potential. We hope to establish certain morphologic criteria which will serve as reliable indicators in assisting the pathologist to identify those meningiomas associated with ominous biological activity and guarded prognosis.

488. EVALUATION OF PAPILLARY TUMORS OF VENTRICULAR SURFACES OF THE BRAIN

Armbrustmacher, V. W., U.S. Dept. of Defense, Armed Forces Inst. of Pathology, *c/o Walter Reed Medical Ctr., Washington, District of Columbia,* 20306, United States of America

The purpose of the study is to determine the histologic criteria for distinguishing between a choroid plexus papilloma and papillary ependymoma. In addition, we would like to determine whether there is a difference in prognosis and biologic behavior of the two neoplasms.

We are continuing to evaluate tumors which fit the histologic criteria for choroid plexus papilloma or papillary ependymoma. We have found that there is a population of tumors which have features of each type of neoplasm. We are in the process of gathering follow-up information to see if there is a difference in biologic behavior. Well-fixed tissue from each type is needed for use in electron microscopic studies.

489. PILOCYTIC ASTROCYTOMA OF CEREBELLUM

Henry, J. M., DeGirolami, U., Earle, K. M., Smith, M. T., U.S. Dept. of Defense, Armed Forces Inst. of Pathology, *c/o Walter Reed Medical Ctr., Washington, District of Columbia,* 20306, United States of America

Pilocytic astrocytomas represent astroglial neoplasms associated with a characteristic histologic pattern; typical sites of origin include cerebellum, optic nerve and infundibulum. These neoplasms occur most frequently in children and young adults, and are often associated with an excellent prognosis following surgical removal.

We have reviewed approximately 80 cases of cerebellar pilocytic astrocytomas. Clinical information and various histologic parameters are ready for computer tabulation; follow-up information has been completed. Computer analysis will be utilized to determine which clinical and histologic features are of prognostic significance.

It is our impression, contingent upon detailed computer analysis, that pilocytic astrocytomas of the cerebellum are generally associated with an indolent post-operative course and a prolonged life span. Publication will conclude multifactorial analysis of clinical and histologic patterns, ultrastructural characteristics, and overall prognosis.

490. HISTOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF OLIGODENDROGLIOMAS

Smith, M. T., Henry, J. M., Earle, K. M., Armbrustmacher, V., Helwig, C., U.S. Dept. of Defense, Armed Forces Inst. of Pathology, *c/o Walter Reed Medical Ctr., Washington, District of Columbia,* 20306, United States of America

The purpose of this study is to ascertain the histological characteristics of oligodendrogliomas and their frequency; biological behavior of oligodendrogliomas; and correlation of biological behavior with histologic characteristics, if any exist.

The study entails clinical follow-up and histological review of 550 cases of oligodendroglioma in the files of the Institute. Review of these cases is proceeding well.

The histological review is nearing completion. The case follow-up is almost complete. Preliminary results will be presented at the next annual meeting of the American Association of Neuropathologists.

491. CLONAL ANALYSIS OF A VERTEBRATE CENTRAL NERVOUS SYSTEM

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This work aims to map the spatial distribution of the clones of cells in the central nervous system (CNS) that are descendants of identified founder cells in the 2 to 128-cell frog embryo. Founder cells will be labelled by intracellular injection of horseradish peroxidase (HRP) which will be identified histochemically in the descendant of the injected cell. Intermediate stages in the deployment of the clone of labelled cells in the CNS will be made, especially of the cells that we have shown to cross the midline and enter the neural retina as a coherent clone. The degree of segregation or of mingling of cells in the CNS of different clonal origin will be elucidated. The experi-

ments also aim to discover when the first embryonic cells originate that contribute their progeny exclusively to the CNS. The relationship of clonal origin to development of a specific neuronal phenotype will be investigated by tracing the lineage of Mauthner's neuron from early embryonic stages, and by determining the variability of that lineage. Preliminary experiments have been done which show the feasibility of the proposed experiments. The preliminary finding that an incipient optic chiasm develops due to translocation of cells across the midline of the embryonic forebrain provides a possible mechanism for the misrouting of optic axons that occurs at the chiasm in albinos (including man). Another important practical application of this work is to provide a basis for a classification of CNS tumors, especially neoplasms of embryonic origin, based on their clonal origins.

E. OTHER BIOLOGICAL AND BIOCHEMICAL STUDIES

492. BIOLOGICAL PROPERTIES OF FETAL BRAIN CELLS DURING MALIGNANT TRANSFORMATION IN CELL CULTURE

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OBJECTIVES: a) Investigation of phenotypic properties of fetal rat brain cells during the gradual malignant transformation in cell culture. b) Use of malignant neurogenic cell lines for experimental therapy.

APPROACH: The project was started in Professor M. F. Rajewsky's group in Tubigen and is continued in close cooperation with his institute in Essen, Germany. Pregnant rats, 18th day of gestation, are given a single pulse of ethylnitrosourea, which gives a very high yield of neurogenic tumors in the offspring. Shortly after the in vivo carcinogen pulse, the brains of the fetuses are transferred to cell culture, where they undergo gradual malignant transformation until they are tumorigenic after a period of 200 days. The differentiation of neoplastic and pre-neoplastic cells is studied using different nervous system specific markers. Karyotyping and measurement of cellular DNA content are performed in parallel.

PROGRESS: The experimental system has been reported on earlier and results from detailed electron microscopic analysis of the same sequence of transformation are published in the same journal. Different classes of neural cells which participate in the process of neoplastic transformation have been identified by transmission electron microscopy. Concomitant alterations of cytoskeleton have been investigated in parallel. Expression of different nervous system specific markers is under investigation.

Cytostatic effects of different platinum compounds on such neoplastic cell lines have been investigated under in vivo and in vitro conditions. Combination therapy with cytostatic drugs and hypothermia is in progress.

493. EMBRYOLOGIC CONTROL OF CANCER

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When blastocysts of the mouse, which have been injected with a few embryonal carcinoma cells, are placed in the uteri of pseudopregnant carrier females, chimeric mice are born. These animals behave normally, and the conclusion is reached that the blastocyst is capable of regulating the malignant cells. We have developed an assay to determine the mechanism by which the blastocyst regulates embryonal carcinoma cells. The blastocyst cannot regulate melanoma cells.

Since the experiment with melanoma cells suggests that there may be a degree of specificity in the ability of embryonic fields to regulate cancer cells, C1300 neuroblastoma cells, parietal yolk sac carcinoma cells, L1210 leukemia cells, and sarcoma 180 cells will be injected in the blastocyst and tested for tumorigenicity. If they are not regulated by this embryonic field, a search will be made for the appropriate regulating embryonic field. For example, neuroblastoma cells might be regulated by the environment of the primitive neural tube, or of the developing neural crest.

494. CELL JUNCTION FORMATION AND ROLE IN CELL PROLIFERATION

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We will continue our study of formation of gap junctions in the following culture systems: Novikoff hepatoma, C6 glioma, BHK fibroblasts, and B-16 mouse melanoma. Electrophysiological, ultrastructural, and tracer techniques will be used to investigate these systems. We will also continue our purification of gap junction proteins and attempt to produce antibodies to them. Finally, we are studying the physiological roles of gap junctions in hormone-induced responses and in mutual growth regulation in mixed cell cultures.

BIBLIOGRAPHIC REFERENCES: J.D. Sheridan, M. Hammer-Wilson, D. Preus, and R.G. Johnson (1978) Quantitative analysis of low-resistance junctions between cultured cells and correlation with gap-junctional areas. J. Cell Biol., 76:532-544.

495. CATECHOLAMINE METABOLISM IN FAMILIAL PHEOCHROMOCYTOMA

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OBJECTIVE: The investigation of the mechanism of normal blood pressure and the aging process in patient and familial pheochromocytoma. This project includes: a) Platelet uptake of catecholamines; b) COMT and MAO activity, RBC and tumor tissue; c) pressor responsiveness to I.V. NE infusion; and d) autonomic nervous system evaluation of these patients and control subjects.

496. EYE STUDIES WITH RADIONUCLIDES

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This is part of a broader project. A summary of this subproject is not available.

497. BRAIN TUMORS AND CONGENITAL MALFORMATIONS (RAT)

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This is part of a broader project. A summary of this subproject is not available.

498. RESISTANCE TO INTRACEREBRAL INOCULATION OF CANCER CELLS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

499. KINETICS OF HUMAN BRAIN TUMORS (MAN, RATS)

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This is part of a broader project. A summary of this subproject is not available.

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